THE EFFECT OF DIETARY FATTY ACIDS ON BODY COMPOSITION, GROWTH, MORTALITY AND SALTWATER TOLERANCE IN JUVENILE COHO SALMON (Oncorhynchus kisutch)

by

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ABSTRACT

An experiment was designed to determine whether the response of fish to graded dietary levels of essential fatty acids (n3) was affected by the total dietary lipid concentration. Juvenile coho salmon were fed practical diets varying in lipid source and total lipid content. The diets were fed in duplicate during a 27-week and a 12-week period ran in succession. The effects of the different dietary fatty acid concentrations on body fatty acids composition were determined after each period. The dietary fatty acid classes were expressed either as percent of the dry diet or percent of the dietary lipid. Analysis of the body lipid fatty acid composition was performed for neutral and polar lipid fractions. The effect of dietary fatty acid concentration on growth and mortality was determined over a 12-week growth study (period 2). A 24-hour saltwater challenge was performed at the end of period 2. It was used to examine the effect of dietary fatty acid concentration on saltwater tolerance.

Dietary n6 and n3 fatty acids appeared to be selectively incorporated into the body polar lipid pool. Linoleate and linolenate underwent elongation and desaturation which resulted in the inhibition of the elongation and desaturation of 18:1n9. The neutral lipid

pool served as a source of n3 fatty acids for the polar lipid when dietary intake was limited by low temperatures during period 1. The body neutral monounsaturated and 18:1 monounsaturated fatty acids consistently reflected the composition of the diet. The n3 fatty acid concentration in the neutral lipid was also directly related to the dietary fatty acid composition during period 2.

The effect of dietary fatty acids on the body neutral or polar fatty acid composition did not depend on the manner in which the dietary fatty acids were expressed. There was also no significant effect of dietary total lipid concentration on the relationship between dietary fatty acids and their incorporation into the body lipids.

The growth response was difficult to interpret because of the high mortality. There was a significant difference in mortality among treatments. A positive relationship between dietary concentrations of total n3 fatty acids or n3 highly unsaturated fatty acids and mortality became evident following analysis of the regression of mortality as a function of dietary fatty acid composition. The dietary fatty acid composition did not appear to alter the saltwater tolerance of the 1+ coho salmon.

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Introduction

The farming of salmon is a vivid example of history repeating itself. The salmon being consumed by the world is increasingly becoming the result of a shift in man's role from that of a hunter to one of a keeper of domesticated creatures. In this modern day move towards civilization aquatic animals and plants are being removed from their natural habitat and raised with some degree of intensity. Mankind has often prospered as a result of its ability to perform this transition effectively. It is this art of manipulating natural systems that allows man to truly exploit his environment. The act of effectively transferring an organism from its natural environment to an artificial environment is referred to as "domestication". The effectiveness of this transfer refers to the ability of the organism to survive, reproduce and grow in its artificial environment.

Understanding the creature is essential to the act of domestication. Initially, the understanding is essential only to propagate the species. However, refined knowledge eventually leads to greater efficiency in manipulating the creature. Behaviour, locomotion, reproduction, disease resistance and nutrition are general areas of study.

Within each of these areas are numerous fields of study.

The study of salmon nutrition has been logically separated into the fields of protein, lipid, carbohydrate, moisture, vitamin and mineral nutrition. Within each of these fields lie specific areas of inquiry which either ask a question of immediate relevance or are aimed at further understanding the animal in question.

Lipid nutrition in salmon is studied for both of the above reasons. Questions which are pertinent to the present fish farming industry involve growth maximization, limiting mortality, and the maintenance of organoleptic properties in the fish. Broader areas of interest within this field involve the understanding of the metabolism of lipids and other nutrients in the body.

The study of fatty acids within the dietary lipid also address specific questions and general knowledge. Pertinent questions involve the relationship of growth and mortality to dietary fatty acid concentrations. Are there any constituents of dietary lipid which maximize growth or minimize mortality? Is diet palatability affected by dietary lipid composition or total content? Do different species of salmon react differently to dietary lipids? Are the different stages of the salmon's life cycle affected similarly by dietary fatty acid composition?

The practical importance of studying the effect of dietary fatty acids on coho salmon

Present estimations of world marine fish oil stores would be sufficient to meet British Columbia's aquacultural needs in feed formulation. However, increased demand of high quality marine fish oils may be seen in the human food and medicine sector as their ameliorating effects of n3 highly unsaturated fatty acids is further documented.

The residual oil in fish meal is a significant source of n3 fatty acids. Mugrditchian et al. (1981) calculated the n3 content in herring meal to be approximately 6.7 % of the residual oil. The reported level equals approximately 0.6% of the meal itself on a dry matter basis. Changes in fish meal quality and availability may affect the n3 content in fish diets.

It is crucial that the quality of primary ingredients in fish feeds is consistently maintained. Unfortunately, fish oils can vary dramatically in quality. Quality of raw ingredients, processing conditions and procedures, the use of stabilizing agents, storage and handling are factors which dictate the quality of the end product. The quality of the fish oil will affect its fatty acid content.

The n3 content of fish varies among species. The fatty acid composition of menhaden oil is not equivalent to the composition of herring oil.

Fish oils from the Norwegian herring oil and meal industry intended for use in aquaculture (NorSalmOil) are marketed with a guaranteed content of less than 4.5% free fatty acids. It is assumed that oils with a high free fatty acid content have probably been exposed to conditions which may result in high levels of oxidization. Austreng and Gjefsen (1981) found crude (unrefined) capelin oils containing 1.8 - 11.0 % free fatty acids. Variances such as these in a relatively unregulated commodity create concerns for fish oil based fish diets.

As increased demand calls on a world market place to provide fish oil, fish feed manufacturers could see great variation in the quality of fish oil based diets. As a precautionary step, feed manufacturers would be wise to decrease their reliance on fish oils and spread the nutritional responsibility of the lipid source to alternate oils in combination with marine fish oils. The incorporation of multiple ingredients into diets provides the manufacturer with the flexibility of diet manipulation. Subtle changes in diet composition should not result in drastic changes in growth, feed efficiency

and survival providing nutrient requirements have been met.

To date only a few alternate lipid sources have been used in salmonid diets. Yu et al. (1977). used swine fat as an energy source in trout rations. Neither intake nor feed efficiency was affected by the inclusion of lard to a maximum of 50% of the total lipid. Takeuchi et al. (1978) implemented hydrogenated fish oil and beef tallow as a dietary energy source for carp and rainbow trout. They indicated that alternate oils can effectively serve as energy sources once the essential fatty acid requirement has been met. Cowey et al. (1979) effectively replaced a portion of the marine oil in a rainbow trout with hard fat from partially rendered hide fleshings without adversely affecting growth. Cho et al. (1974) compared soybean oil, rapeseed oil or marine oil in rainbow trout diets. Weight gain in the fish was not affected by lipid source. However, feed conversion was significantly better for fish on the marine oil diet. Mugrditchian et al. (1981) used linseed oil and animal fat as alternative lipid sources in dry diets for chinook salmon (Oncorhynchus tshawytscha). From the lack of a significant dietary effect in each example we can conclude that once the essential fatty acid requirement has been met alternative lipid sources can

effectively be utilized in the diet provided that digestibility is consistent among lipids.

Differences in the growth response to different fatty acids among the salmonids may be further justification for the use of multiple lipid sources. Dosanjh et al. (1988) evaluated canola oil, pork lard and marine lipid, singly and in combination, as supplemental dietary sources for juvenile chinook salmon (Oncorhynchus tshawytscha). This preliminary report found that diets using alternate lipid sources or combinations of sources result in significantly better feed conversion than do diets using only herring oil. There were no differences in growth response among the alternate lipids or their various combinations. Dosanjh et al. (1984) reported on the efficacy of canola oil, pork lard, and marine oil singly, and in combination, as supplemental dietary lipid sources for juvenile coho salmon (Oncorhynchus kisutch). The lack of dietary influence in this salmonid species suggests, as is the case with other salmonids, that alternate lipid sources which supplement an adequate content of essential fatty acids do not impede growth or feed conversion. However, remarks were made regarding the palatabilty and texture of diets and the effect that alternate lipids might have on these two important dietary traits.

The digestibility of alternate lipid source must be considered when dietary manipulations occur. Takeuchi et al. (1978) reported that the digestibility of highly hydrogenated oils was seen to be 20% lower than a combination of soybean and cuttlefish oil. The digestibility of the hydrogenated oils mixed with n3 highly unsaturated fatty acid source oils was not drastically lower than that of the n3 highly unsaturated fatty acid oil alone while the digestibilty of hydrogenated fish oil was markedly lower.

Studying the essential fatty acid requirement in fish

Fatty acid research in salmon also addresses the comprehensive questions of which fatty acids are essential to that animal, at what levels, and why. Understanding these important principles will allow nutrition research to progress effectively in its attempt at resolving the daily dilemma of aquacultural endeavors.

Initially, lipid nutrition and fatty acid metabolism in salmonids were accepted as being similar to those of terrestrial animals. However, this extrapolation proved to be erroneous. Terrestrial animals and fish do share the inability to elongate and desaturate specific fatty

acids. Polyunsaturated fatty acids cannot be synthesized de novo in either case. The inability to synthesize these compounds defines the essentiality of the fatty acids. However, the idea that different families of fatty acids (linoleic or linolenic) might be required for different animals was not embraced until after until early researchers, Phillips et al. (1963), unknowingly reported the importance of n3 polyunsaturated fatty acids in salmonids. They found that the addition of corn oil to a diet was limited in its effect when fish meal was deleted from the diet of brook trout (Salvelinus fontinalis) growth. Fish meal was the only significant source of n3 fatty acids in the diets. This initial naive observation has resulted in the statement of n3 essentiality in teleosts. It has also led to much debate regarding the roles of n3 and n6 fatty acids in terrestrial animals and fish respectively.

The field of a fatty acid research in fish has primarily involved salmonids. It is erroneous to extrapolate these results across species. The limited research that has involved other species of fish has found differences between species. Yamada et al. (1980) has demonstrated that 18:3n3 is essential to rainbow trout (Oncorhynchus mykiss), carp (Cyprinus carpio) and the eel

(Anguilla japonica). This fatty acid is of much lesser importance to marine fishes such as Red sea bream (Chrysophrys major), Black sea bream (Mylio macrocephalus), opaleye (Girella nigricans), and freshwater fishes such as yellowtail (Seriola quinqueradiata). It was assumed that the difference in essentiality lies in the ability to convert 18:3n3 to n3 highly unsaturated fatty acids. From these reports it is assumed that there is much variation in essentiality of fatty acids. Elongation and desaturation of fatty acids is thought to occur in other species. However, the rate of this conversion does seem to vary from species to species.

The mechanisms of lipid metabolism and biosynthesis seem to be universal among teleosts (Yamada et al 1980). All species which possess the appropriate enzymes do convert some short chain n3 fatty acids into longer chained polyunsaturates as well as into shorter, more saturated fatty acids. 18:3 n3 can be broken down through B-oxidation and new fatty acids biosynthesized through acetyl-CoA.

Some teleosts are unable to elongate and desaturate short-chained n3 fatty acids. Owen et al. (1975) discovered that the turbot (Scophthalmus maximus L.) is

one such species. Using radioactive tracers they showed that the turbot require long-chained highly unsaturated fatty acids while rainbow trout are able to elongate and desaturate 18:3n3 to n3 highly unsaturated fatty acids.

Elongation and desaturation of both the linolenic and linoleic family fatty acids occurs in the rainbow trout when the respective diets were fed (Castell et al. 1972c). The elongation and desaturation mechanism for producing >C20 fatty acids seems to be common to n3, n6 and n9 fatty acids. Yu and Sinnhuber (1972) observed that 20:3n9 was biosynthesized in rainbow trout from n3 deficient diets. The elongation and desaturation of non-essential fatty acids was decreased when dietary n3 levels were increased thereby providing evidence for the idea of substrate dependent elongation and desaturation.

It was evident to Yu and Sinnhuber (1979) that both n6 and n3 fatty acids are able to inhibit the incorporation of 20:3n9 into body phospholipids in fish. The competition between essential and nonessential fatty acids for elongation resulted in the development of the essential fatty acid index. The essential fatty acid index is a tool used to estimate essential fatty acid deficiency. It is simply the ratio of 20:3n9 /22:6n3. Takeuchi et al. (1979) stated that 20:1n9 and 18:1n9

concentrations in the body decrease when linoleic or linolenic acid is fed. This can be associated with the elongation of n9 fatty acids. Takeuchi and Watanabe (1977a) observed that the addition of linolenate to the diets decreased the amount of 16:1, 18:1 and 20:3 n9 in the body of rainbow trout. However, n3 highly unsaturated fatty acids are more effective in decreasing abnormal levels of the n9 fatty acids. There was a low essential fatty acid index in rainbow trout fed high lipid diets with only 1% linolenate. The acceptable index level occured even though that level of supplementation was not sufficient to maximize growth. Therefore, the essential fatty acid index is not a valid tool for growth maximization. The ratio of 20:3 n9 / 22:6 n3, which has been used to indicate the adequacy of dietary lipids in rainbow trout, cannot be used in turbot because of turbot's inability to elongate oleic acid according to Owen et al. (1975). This would apply to other marine fish with similar n3 elongation and desaturation capacities.

The salmonids

Differences in life cycle and diet are the two most obvious reason why differences in fatty acid nutrition in

these fish might be expected. It can be expected that species which have evolved under a specific environment and dietary regime might metabolize fatty acids differently. Rainbow trout are freshwater residents while coho (Oncorhynchus kisutch), chum (Oncorhynchus keta), chinook (Oncorhynchus tshawytscha), sockeye (Oncorhynchus nerka), pink (Oncorhynchus gorbuscha), Atlantic (Salmo salar) and steelhead salmon (Oncorhynchus mykiss) all are anadromous under different life cycles and environments. Diet does differ within and among species due to these differences.

Within salmonids the n3 fatty acids requirements do differ from species to species. Takeuchi and Watanabe (1982) found species differences in the effect of short chained and long-chained n3 polyunsaturated fatty acids on rainbow trout, coho salmon, and chum salmon. Hardy et al. (1987) reported that Atlantic salmon respond to dietary fatty acids in a manner similar to Pacific salmon and rainbow trout. Specific differences will be expanded on later in the text.

The essentiality of n3 fatty acids in salmonids

A compound is deemed to be nutritionally essential if

it is necessary to maintain life and cannot be sufficiently synthesized de novo in the body. n3 fatty acids were defined as essential in rainbow trout by Castell et al. (1972a, 1972b, 1972c). Diets deficient in n3 fatty acids resulted in mortality, fin erosion, pale enlarged livers, increased mitochondrial respiration, acute myocarditis, mild macrocytic hypochromic anemia and "fainting" or shock syndrome and poor growth.

The linolenic family of fatty acids were exclusively responsible for the correction and prevention of the deficiency symptoms. The inclusion of 1% linolenate in the diet of rainbow trout corrected mitochondrial swelling, prevented the shock syndrome, decreased mortality, improved growth and completely prevented fin erosion.

It has been proven that n3 fatty acids are essential in preventing the maladies described above. However, the actual mechanism by which these essential nutrients correct and prevent these symptoms still eludes researchers. What is it about this group of fatty acids that makes them essential to teleosts? The roles that these compounds might play in the physiology of the fish will be discussed later.

The site of the double bond with respect to the terminal methyl group does not seem to be the exclusively responsible for the definition of essentiality. Takeuchi and Watanabe (1982) failed to meet the n3 requirement of rainbow trout, coho salmon and chum salmon using ^12,19-C22:2. This nonmethylene interrupted (NMID) polyethylenic fatty acid possesses a double bond at the n3 position but has another near the carboxyl end. It is evident that the definition of n3, a carbon-carbon double bond situated between the third and fourth carbons on a chain, is not the sole reason for essentiality.

The melting point of fatty acids has been associated with their essentiality in poikilotherms. It would seem physiologically unsound for the fish to incorporate fatty acids with high melting points into the body.

The melting point of fatty acids usually decreases as the degree of unsaturation increases. The melting point of C18 fatty acids decreases from +16.3^{o}C in oleic acid (18:1 n9) to -5.0^{o}C in linoleic acid (18:2 n6) to -11.3^{0}C in linolenic acid (18:3 n3). Parinaric acid (18:4n3) is an exception to the rule having a melting point of +84^{o}C. The high melting point in this case is due to the structure of compound. It is uncertain whether poikliotherms incorporate fatty acids differently at

different ambient temperatures. For this reason it is important to consider water temperature when fatty acid nutrition experiments are conducted.

The fact that both linoleate and linolenate have melting points below the lethal temperature for teleosts does not answer the question why n3 and not n6 fatty acid are essential in salmonids. The whole issue of whether or not n6 fatty acids are actually essential in salmonids will not be fully addressed in this paper. Essential nutrients may be required in such small amounts that even purified diets may provide adequate amounts (Yu et al. 1979).

The Roles of n3 fatty acids

Essential fatty acids as prostaglandin precursors

The most likely essential role of n3 fatty acids in teleosts involves prostaglandin synthesis. The role of essential fatty acids in prostaglandin synthesis in terrestrial animal leads nutritionists and biochemists to hypothesize a similar role for the n3 family of fatty acids in fish. However, current research has been unable to identify a biosynthesis system that is different than

that of terrestrial animals. The main precursor fatty acid in these animals is arachidonic acid (20:4n6). 20:3n6 and 20:5n3 are the next most important precursors.

Prostaglandins have been detected in finfish and lower animals. Nomura and Ogata (1976) isolated prostaglandins in carp, sheat-fish, leopard shark, as well as in crawfish, blue crab, mussel, scallop, sea-squirt, and sea-anemone. It appeared to these researchers that the gastro-intestinal tissues, air bladder, heart and gill of fishes are inclined to have a higher level of prostaglandin than other tissues.

It is assumed, but not confirmed, that prostaglandins in fish are important in modulating numerous important physiological functions including cardiac, pulmonary, and muscular. It is also uncertain whether or not the fish contains prostaglandins in addition to those found in terrestrial animals. Prostaglandins of the 1, 2, and 3 series are synthesized from the C20 unsaturated fatty acids eicosatrienoic acid (20:3 n6), eicosatetranoic acid (20:4n6) and eicosapentanoic acid (20:5n3) respectively. In terrestrial animals the 2 series prostaglandins are predominant. Anderson et al. (1981) suggested that the mechanism for PGE_2 in fish is similar to that found in higher vertabrates.

In an preliminary report Mai et al. (1981) announced the discovery of a new prostaglandin C22-$PGF_{4\alpha}$, synthesized from docosahexaenoic acid (22:6n3) by trout gill. The presence of several prostaglandins with three double bonds in the gill of rainbow trout was also reported. In addition significant amounts of an unknown compound with chain length equivalence of 22 carbons was observed. The evidence for the presence of a $PGF_{4\alpha}$ was disputed by the laboratory which initially made the report. German et al. (1983) communicated that the predominant oxygenating enzyme in trout gill tissue is a lipoxygenase enzyme and the compound previously described by Mai et al. (1981) as $PGF_{4\alpha}$ is actually trihydroxylated compounds of the hydroperoxyfatty acids generated by the action of the lipoxygenase. It was concluded that current evidence suggests that trihydroxy polyenoic fatty acids are predominant products and n3 prostaglandins are apparently not produced in quantity by trout gill tissue. The search for series 3 prostaglandins in marine fish once again was unfruitful when Anderson et al. (1979) found that PGE_2 was the only prostaglandin identified in vitro in plaice, Pleuronectes platessa L., regardless of the significant concentration of 20:5n3 in the tissue cultured.

Much of the research in fish prostaglandin synthesis has been carried out in vitro on rainbow trout. It was not stated, but, it is assumed that these were freshwater fish. This fact may be of significance when the osmoregulatory mechanisms of the fish are considered. Anadromous fish, of which salmonids are definitely included, develop chloride cells in the gill tissue at smoltification (immediately prior to entering the salt water habitat). Research involving marine fish may demonstrate the presence of prostaglandins in the fish gill which are exclusive or more important in the fish. Bell et al. (1983) described the importance of n3 and n6 polyunsaturated fatty acids in the salt secreting epithelia of two marine fish species. The turnover of phosphatidylinositol in the gills of seawater eels (Anguilla anguilla) was elicited by an α-adrenergic stimulus, a condition known to inhibit salt secretion by the gills. Phosphatidylinositol is also involved in prostaglandin formation by being the source of the 20:4n6 precursor used for $PGE_{2\alpha}$ synthesis. PGE_2 is known to inhibit salt secretion by marine teleost gills (Pic, 1975).

There is evidence that 20:4n6 rather than eicosapentaenoic acid is the preferred precursor of

prostaglandins in marine fish. Anderson et al. (1981) found arachidonate to be the only significant prostaglandin precursor of the essential fatty acid precursors for series 1, 2, and 3 prostaglandins in plaice skin. The concentration of the precursor in the fish did not seem to effect prevalence of the corresponding prostaglandin. In the plaice the eicosapentaenoic acid concentration was approximately three times that of arachidonaic acid. However, the conversion of eicosapentaenoic acid to PGE_3 was approximately 7 times less than that of 20:4n6 to PGE_2.

The importance of phosphatidylinositol and more specifically 20:4 n6 may redefine the essential fatty acids in fish. The fact that phosphatidylinositol is present in a very minute quantity in the fish probably makes the requirement levels of arachidonate in the diet extremely low.

While eicosapentaenoic acid has not been found to be important as a prostaglandin precursor it is likely that this essential fatty acid plays a regulatory role in prostaglandin synthesis in fish. This was observed in the plaice skin by Anderson et al. (1981). This effect is evident in human medicine by the ability that eicosapentaenoic acid has in inhibiting the conversion of

20:4n6 to thromboxane A_2, which is an important aggregator of platelets. Platelets seem to have a limited ability to metabolize eicosapentaenoic acid.

Essential fatty acids in biological membranes

Essential fatty acids from the diet can be incorporated into the polar lipid which is found throughout the body. These polar lipids play an important role in maintenance of the integrity and function of membranes according to the current understanding of biological membranes.

Castell et al. (1972b) observed that the flesh of rainbow trout fed a fat-free diet were abnormally soft and flaccid in comparison to fish fed linolenic acid. Decreasing the level of linolenic acid in the diet apparently promoted an increase in the muscle water content. If the effect is real, it would suggest that the change in muscle composition may be due to alterations in membrane permeability as a result of the lack of n3 fatty acids. Takeuchi and Watanabe (1979) observed a high body moisture content in rainbow trout fed a diet containing 4% methyl linolenate. Once again, manipulating the dietary n3 content may result in changes in membrane permeability.

Essential fatty acids neurological function

The "fainting" or shock syndrome observed in n3 fatty acid deficient fish suggests that these fatty acids play a neurological role in the fish. Singh and Chandra (1989) reviewed literature which showed substantial amounts of 22:6n3 in gray matter, white matter, myelin, synaptosomal plasma membrane and synaptic vesicles in the brains of humans, the mouse and the rat. The highly unsaturated fatty acids seem to be maintained in the polar lipid in the salmonid brain tissue even after spawning (Phleger et al., 1989). The above factors strongly suggest that n3 highly unsaturated fatty acids are essential components of brain lipid in salmonids.

The effect of dietary fatty acids on body fatty acid composition

The body polar lipid

The lipid composition of different body organs seem to be affected differently by dietary fatty acid composition. Observations regarding total lipid fatty acid concentration are not as illuminating as are observations based on the fatty acid content of the

neutral or polar fractions. Polar lipids, phospholipids, are considered as structural while neutral lipids are considered primarily as body lipid reserves.

Body organs differ in their relationship to dietary fatty acids. Much of these differences relate to the ratio of polar lipid to neutral lipid in that organ. The liver is a source of lipid synthesis and modification. The concentrations of neutral and polar lipid depends on the state of level of synthesis. The brain, having more polar lipid than neutral, retains a relatively stable fatty acid profile which is intolerant to changes in dietary fatty acid composition. The muscle fatty acid composition, because of its higher neutral lipid content, usually reflects the diet.

The brain polar lipid does not seems to be equally responsive to linoleate and linolenate (Castell et al. 1972c). The polar lipid does remain consistantly high in highly unsaturated n3 fatty acids regardless of dietary fatty acid content. Similar findings were reported by Takeuchi and Watanabe (1977b).

The lipogenic nature of the liver does not prevent it from being affected by dietary fatty acids. The fatty acid content of this organ is affected by dietary lipid. Castell et al. (1972c) found the highly unsaturated fatty

acids in the polar lipid to be more responsive to dietary fatty acids than was the neutral lipid. Takeuchi and Watanabe (1982) reported that in rainbow trout and coho salmon the liver total lipid and nonpolar lipids were influenced by diet. However, liver polar lipid in chum salmon was not affected by dietary lipid content. Takeuchi and Watanabe (1982) observed the substantial concentrations of 20:3n9, 16:1 and 18:1 in the polar lipid of livers in rainbow trout fed n3 deficient diets. These fatty acids are products of the non-essential fatty acids provided in the diet.

Dietary fatty acid concentration also affected the fatty acid profiles of heart and kidney lipid fractions (Castell et al. 1972c).

Although the phospholipid fraction fatty acid content is less reflective of dietary content than the neutral fraction it is still affected by dietary changes. Castell et al. (1972c) found that the n3 content in the phospholipid fraction of the rainbow trout body lipid was increased when the dietary n3 level increased to 1% on a dry matter basis (DMB).

n3 fatty acids are selectively incorporated into the phospholipid as highly unsaturated fatty acids when suboptimal concentrations of essential fatty acids are

fed. Deficient diets result in the incorporation of highly unsaturated non-essential fatty acids in the polar lipid. It is believed that the mechanism for elongation and desaturation of fatty acids in fish is the same for n3, n6 and n9 fatty acids. The rates of elongation and desaturation are most likely substrate dependent. It has been suggested by Brockerhoff et al. (1966) that 22:6n3 inhibits the incorporation of 20:3n9 into the B position of the glycero phospholipids. As a result formation of 20:3n9 was limited.

Therefore it is believed that n3 highly unsaturated fatty acids are selectively incorporated into the polar lipid without being metabolized. Short-chained n3 fatty acids from the diet are desaturated and elongated on a substrate concentration basis. They, in turn, are incorporated into the polar lipid selectively when they are in the form of long-chained highly unsaturates.

Further evidence of the selective incorporation of n3 fatty acids was reported by Yu and Sinnhuber (1979) and Castledine and Buckley (1980). They found that the proportion of 22:6n3 incorporated into body phospholipid from the diet increases as the concentration in the diet decreased.

Non-deficient fish incorporate n3 fatty acids into

the polar lipid at rates which reflect the dietary concentration (Yu et al. 1979). This relationship is not exclusive to the linolenate family of fatty acids. Lee et al. (1967) noted that the body phospholipid fraction of fish contained low levels of 22:6n3 and high levels of 22:5n6 when the diet was composed primarily of linoleic family of fatty acids. Castledine and Buckley (1980) concurred with this observation. However, Yu and Sinnhuber (1979) discovered that high concentrations of 18:3n3 in the diet decrease the incorporation of n6 fatty acids into body phospholipids. High dietary n6 concentrations did not affect the incorporation of n3 into body phospholipids.

Long-chained polyunsaturated fatty acids are preferentially incorporated into the phospholipid fraction. Lee et al. (1967) found that diets containing n3 highly unsaturated fatty acids resulted in a higher concentration of 22:6n3 in the body polar fraction than diets containing 18:3n3 as the only n3 source. The preference for long-chained highly unsaturated fatty acids involved n6 as well as n3 fatty acids (Takeuchi and Watanabe 1982).

Body polar fatty acids other than the n3 and n6's do not seem to be affected by dietary content. Castledine

and Buckley (1980), found that the percentage saturated fatty acids remained constant within the phospholipid pool regardless of the diet lipid source.

It is uncertain whether or not dietary fatty acid composition affects the amounts of polar lipid in the body. Castledine and Buckley (1980) stated that the size of the phospholipid pool relative to body weight in rainbow trout does not vary with changes in n3 fatty acid content of the diet. It remained relatively constant at 0.97% wet body weight. However, Castell et al. (1972c) observed that the amount of phospholipid in the body was associated with dietary levels of linolenic acid.

Body total lipid and neutral lipid

Fatty acid composition based on total body lipid is difficult to interpret because of the lack of homogeneity in the lipid fractions. The polar and neutral lipid fractions are not found in the body in equal amounts. These two fractions also react differently to dietary fatty acid concentrations. The polar lipid is somewhat selective while the neutral lipid masks the diet composition. The diet will tend to affect the total body lipid in a manner similar to the neutral lipid simply

because of the relative abundance of that fraction.

Increasing dietary linolenate does change body fatty acid content (Takeuchi and Watanabe, 1977a). The addition of linolenate to the diets decreased the amount of 16:1, 18:1 and 20:3 n9 in the body of rainbow trout. Castledine and Buckley, (1980) found that in rainbow trout the percentage saturated fatty acids increased in the body neutral lipid fraction with increases in dietary saturate content. The n6 concentration in both the polar and the neutral lipid fractions were also affected by changes in the dietary fatty acid profile.

Castledine and Buckley (1980) observed what appeared to be an inverse relationship between dietary n3 content and body 22:6n3. The form in which ingested dietary fatty acids, especially n3 fatty acids, are incorporated into the neutral lipid depends on the ability of the animal to desaturate and elongate fatty acids. Fish that are unable to desaturate and elongate fatty acids will possess neutral lipid fractions which closely match the profile of the dietary lipid (Yamada et al. 1980). This difference in fatty acid metabolism is the primary reason for differences in the neutral lipid fatty acid profile among fishes.

Increases in the neutral lipid pool are seen during

periods of positive energy balance. These increases are enhanced by dietary lipid, modified dietary lipid or endogenously synthesized lipid. Synthesis and modification would likely occur in the liver because mesenteric adipose tissue has a much lesser lipogenic ability than hepatic tissue. There is no turnover of the neutral lipid pool during this time.

The effect of energy balance on body fatty acid composition

During periods of negative energy balance, fatty acids in the neutral lipid are catabolized and are probably unavailable to the phospholipid pool. Castledine and Buckley (1980) observed that starvation caused a reduction in the 14:0, 16:1n7, 18:1n9 concentrations and an increase in the 18:2n6 and 22:6n3 in the phospholipids of rainbow trout except in fish which had received these two fatty acids in large amounts. Castledine and Buckley (1980) hypothesized fatty acids might be catabolized on a molar basis in the neutral lipid. They suggested that 22:6n3 would be catabolized before shorter chain fatty acids. The polar highly unsaturated n3 fatty acids are maintained in times of lipid catabolism. It was unclear whether highly unsaturated n3 fatty acids from the neutral

lipid pool are transferred to the phospholipid pool during periods of catabolism in the body.

Dietary lipids help create a positive energy balance which usually results in growth. The fatty acid themselves and the total fatty acid profile of dietary lipid seems to affect the growth rate and feed efficiency.

The effects of dietary fatty acids on growth

In addition to correcting the classsical essential fatty acid signs, the addition of 18:3n3 stimulates growth and improves feed efficiency (Castell et al. 1972a). Linoleic acid was also found to improve growth and feed efficiency although linolenic acid was superior in this regard (Castell et al. 1977a) and (Takeuchi and Watanabe 1982).

The concentrations and combinations of dietary fatty acids required to enhance growth and feed efficiency seem to vary from species to species. Takeuchi et al. (1979) observed that the addition of either linoleic or linolenic acid improved growth in chum salmon. The best weight gain and feed efficiency was seen when the fish were fed a diet consisting of both 1% 18:3n3 and 1% 18:2n6. Yu et al. (1979) observed weight gain and feed efficiency to be

greatest for rainbow trout fed a 1% 18:3n3 diet. Yu and Sinnhuber (1972) observed that growth and feed efficiency were maximized in rainbow trout when either 1% 18:3n3 or 1% 22:6n3 was fed.

There seems to be no significant difference in growth rate and feed efficiency between diets containing equal amounts of either 18:3n3 or highly unsaturated n3 fatty acids in rainbow trout (Lee et al. 1967). However, Takeuchi and Watanabe, (1977b, 1982) found linolenate to be inferior to eicosapentaenoic acid and decosahexaenoic acid when fed at 0.5% of the diet regarding growth enhancement. A 1:1 mixture of these two highly unsaturated fatty acids proved to be more effective in stimulating growth than they were when fed individually. Takeuchi and Watanabe (1982) did not find much difference in the growth rate of rainbow trout fed 0.5% n3 highly unsaturated fatty acid oil or 1% linolenate. Yu and Sinnhuber (1972) stated that rainbow trout fed diets containing 22:6n3 did not produce significantly better results than did diets containing 18:3n3.

In coho salmon, growth and feed efficiency are maximized when the diet contains 1 - 2.5% 18:3n3 (Yu and Sinnhuber, 1979). Coho respond poorly to diets lacking n3 fatty acids resulting in poor growth (Takeuchi and

Watanabe 1982). It has also been stated that a mixture of n3 highly unsaturated fatty acids fed at 1% of the diet is not effective in improving the growth effect in coho beyond the rate of the fish fed a diet containing linoleate as the only lipid. In that report the researchers concluded that n3 highly unsaturated fatty acids had an adverse effect on growth. It was also observed that 1% 18:2n6 could improve growth caused by an n3 deficiency while 1% arachidonate could not.

Takeuchi et al. (1979) found that the addition of 20:5n3 and n3 highly unsaturated fatty acids in chum salmon diets increased growth beyond the level produced by the addition of the same amount of 18:3n3.

Takeuchi and Watanabe (1982) stated that the best growth was seen in chum salmon receiving a combination of 1% linolenate and 1% linoleate.

The linoleic family of fatty acids are beneficial to growth and feed efficiency in salmonids. There does not seem to be a difference between the response to 18:2n6 or 20:4n6 (Takeuchi and Watanabe 1982). However, there does seem to be some difference between species. In many cases growth is maximized when n3 and n6 fatty acids are combined in the diet.

Takeuchi and Watanabe (1982) addressed the issue of

what effect age and life cycle have on fatty acid requirement. They did not observe any significant difference in the requirement of freshwater and sea water chum. This observation suggests that there may not be a difference in requirements of anadromous fish in their different life stages.

The effect of dietary fatty acids on mortality

High mortaltiy in fish fed n3 deficient diets can be arrested by feeding diets containing the essential n3 fatty acids. Lee et al. (1967) used rainbow trout to show the effect of n-3 fatty acids on mortality. The high mortality found throughout the n3 deficient fish was reduced after 2 weeks of feeding the n3 supplemented diet. Essential fatty acid deficient diets resulted in high mortality in chum salmon (Takeuchi et al. 1979). Mortality was not affected by the addition of 0.5% 18:3n3. In coho, an n3 deficient diet resulted high mortality (Takeuchi and Watanabe, 1982).

The effect of feeding dietary n3 in excess of the requirement

Current research suggests that increasing the dietary

n3 fatty acid content above a minimal concentration improves growth. However, the literature rarely pursues this principle to examine the effect of dietary n3 concentrations which are two or three times the levels used for growth enhancement. Practical salmonid diets which use marine fish oils exclusively will contain such concentrations.

Yu and Sinnhuber (1979) found that the addition of 2.5% linolenin consistently resulted in decreased average final weight and specific weight gain in coho salmon. Feed efficiency was not affected. Takeuchi and Watanabe (1979) discovered that the addition of 4% n-3 fatty acid to rainbow trout diets resulted in poor growth and low feed conversion when compared to diets containing 1% 18:3n3.

The effect of dietary protein to lipid ratio on growth, feed efficiency and body composition

The ratio of protein to lipid in salmonid diets is one area of diet manipulation which should be considered. Being the sources of metabolizable energy these two nutrients become intermeshed. While, like other areas of salmonid nutrition, there is no definitive ratio, some trends have been reported. Unfortunately, these reports

are few and deal primarily with rainbow trout.

Takeuchi et al. (1978a) reported that in the rainbow trout weight gain and feed efficiency improved as lipid increased and was maximum when the diet contained 35% protein and 15 or 20% lipid. Lee and Wales (1973) found improvement in feed efficiency with either an increase in lipid or in protein. Kellems and Sinnhuber (1982) stated that efficiency of feed conversion increased significantly with increases in dietary lipid content. Reinitz et al. (1978) attempted to establish nutrient levels of diets based on a ratio of digestible energy to dietary crude protein. The diet with the greatest energy content provided the best feed efficiency. Unfortunately, the digestible energy (DE) values were based on proximate analysis value which were the same for all ingredients. The authors themselves state that differences such as different amino acid levels could affect digestibility and negate any correlation between DE/CP ratio and protein retention.

The ratio of dietary lipid to dietary protein did affect body composition as was seen by Takeuchi et al. (1978b) and Reinitz et al. (1978). It was suggested that dietary lipid is able to increase protein utilization efficiency. Lee and Wales (1973) documented increases in

liver glycogen levels in rainbow trout on a high protein low lipid diet. These results suggest that when protein rather than lipid is metabolized for energy there is greater biosynthesis of glycogen in the body at liver.

The effect of dietary lipid concentration on growth and body fatty acid composition

A minimum of 10% lipid was deemed necessary to increase the efficiency of protein utilization by Takeuchi et al. (1978a). Takeuchi et al. (1978b) suggested that 10% dietary lipid was sufficient to maintain normal growth in rainbow trout.

Takeuchi et al. (1978b) reported a decreased growth response to lipid levels greater than 20%. Large quantities of lipid such as this may be providing excesses of n6 or n3 fatty acids. On the other hand, increases in dietary lipid might cause an increase in essential fatty acid requirement as a percentage of the dry diet. Takeuchi and Watanabe (1977a) noted that increasing dietary laurate from 4% to 9% or 14% did not alter total body fatty acid composition when 1% 18:3n3 was in the diet.

Simply stating a crude fat requirement either as a minimum level or as a ratio with protein is not

sufficient. After digestibility has been considered the fatty acid profile of the lipid source should be considered. Excesses and deficiencies of both n3 and n6 fatty acids affect performance. Here again is a benefit of multiple lipid sources. Different lipid sources can be selected and incorporated into diets for the purpose of supplying n3, n6 or saturated and monounsaturated fatty acids.

The method by which dietary fatty acid concentrations are expressed

Dietary n3 fatty acid concentrations are not commonly expressed relative to the total lipid content. Concentrations of n3 fatty acids are quantified as a percent of the diet, usually on a dry matter basis. Takeuchi and Watanabe, (1977a) observed that at 5% total lipid, 1% n3 level in the diet produce the best growth response and feed efficiency in rainbow trout. However, when the lipid content was increased to 9 or 14%, using laurate, more than 2% linolenate was required to produce maximum growth. This finding suggests that the dietary requirement for n3 fatty acids might be better stated as a percentage of the total lipid rather than as a percentage of the dry diet. Differences in dietary lipid levels

confound the issue by affecting consumption, energy balance and body lipid storage.

The effect of dietary fatty acid concentration on saltwater tolerance

The effect of dietary fatty acid composition on saltwater tolerance has been addressed by researchers using a 24 hour saltwater challenge. The method for the saltwater challenge has been standardized by Blackburn and Clarke (1987). Mortality and plasma sodium concentrations have been the primary parameters of study. Clarke and Blackburn (1977) have stated that plasma Na concentrations below 170 meq/l indicate saltwater tolerance. Blackburn and Clarke (1987) have stated that mortality tends to be more pronounced when water salinity is greater than 26 ppt. Uno (1989) found mortality to differ between salmonid species.

Markert et al. (1984) noted that plasma Na concentrations in coho salmon (Oncorhynchus kisutch) were not affected by differences in dietary crude lipid content or in dry matter content following a 24 hour saltwater challenge. A similar response was observed by Plotnikoff et al. (1983) in chinook salmon (Oncorhynchus tshawytscha) fed diets of different crude lipid content. Dietary

treatments of 8.16, 12.86, 16.70 and 17.27% crude lipid (DMB) did not produce significant differences in plasma Na concentrations following a 24 hour challenge. In this study the fatty acid the dietary lipid contained 15.4, 21.3, 22.9, or 13.6% n3 fatty acids. These values could be transcribed to n3 % of diet concentrations of 1.26, 2.74, 3.82, and 2.35, respectively. The n6 fatty acid concentrations of the lipid were 10.3, 7.1, 5.5, and 7.1 respectively (0.84, 0.91, 0.92, 1.23 % of diet). The lack of significant difference in plasma Na content suggests that differences in dietary fatty acid concentration does not lead to differences in osmoregulatory ability. However, the mean plasma Na concentrations were above the level indicative of saltwater tolerance.

A second study involving juvenile chinook salmon fed different diets resulted in lower plasma Na concentrations. Plotnikoff (1984) suggested that increased body size could be responsible for the improved osmoregualtion. Unfortunately, statistical analysis was

not used to evaluate the effect of dietary treatments.

Osmoregulation at smoltification seems to be a perfect field of study in the pursuit of the reason for "essentiality" in salmonids. The physiological factors required to overcome the systemic stress of drastic salinity change involve two areas that may involve essential fatty acids, namely prostaglandin synthesis and biological membrane permeability due to composition.

The objective

The study was designed to add to the pool of knowledge required for the effective culture of this aquatic species. However, the practical importance of this research does not detract from its importance in the understanding of lipid metabolism in the fish. The specific objective of the experimet was to determine whether the response of fish to graded levels of essential fatty acids (n3) was affected by the total dietary lipid concentration.

The effect of dietary fatty acids on body composition was studied over two periods. The primary difference between experimental conditions during the two periods was water temperature. The two period scheme was implemented in an attempt to gain a better understanding of this relationship under different physiological states and environmental conditions. The metabolism of essential and non-essential fatty acids were studied by observing the desaturation and elongation products of oleic (18:1n9), linoleic (18:2n6) and linolenic (18:3n3) families of dietary fatty acids within the body.

A large part of the body composition study examined the relationships between body fatty acid composition and

the method by which the dietary fatty acid concentration was expressed. The three methods categorized the diet in terms of the dietary total n3 fatty acids content, the dietary concentration of the fatty acid class being examined in the body lipid (total saturated fatty acids, total monounsaturated fatty acids, total 18:1 fatty acids, total n3 fatty acids, n3 fatty acids longer than C18, total n6 fatty acids, and n6 fatty acids longer than C18), or the method by which the fatty acid was quantified (percent of the dry diet or percent of the dietary lipid).

The relationship between dietary lipid composition and growth or mortality was studied during period 2 of the experiment. This period was defined as the period in which the growth trial occurred. Period 2 began when water temperature began to rise in the spring. The rise in water temperature provided a favorable environment for growth.

A saltwater tolerance study was performed following period 2. The challenge was used to judge the effect of dietary lipid composition on ability of 1(+) year old coho salmon to effectively withstand direct transfer to saltwater. The timing of the study is quite logical as coho salmon commonly undergo smoltification after one year in freshwater.

Materials and methods

Experimental diets

Ten isonitrogenous diets were formulated from practical and purified ingredients (table 1). The diets were formulated to contain 35.0 % crude protein on a dry matter basis (DMB). The crude fiber was calculated as 12.7 % or 6.7 % (DMB) in the low and high lipid diets respectively. The lipids used in each of the diets are listed in table 2. The actual fatty acid composition is summarized on the basis of dry diet (% DMB) in table 4 and on the basis of dietary lipid (% lipid) in table 5. The total lipid level in the diet was 18% or 25% of the total diet (DMB) as is shown in table 3. The higher lipid concentration was achieved by substituting tri-olein for α-cellulose. The diets were isonitrogenous and isocaloric within a lipid level.

The herring oil had been stabilized with the antioxidant ethoxyquin (1,2-dihydro-6 ethoxy-2,2,4-trimethylquinoline) at its source. Additional ethoxyquin was added to the diets through the corn oil. The diets were formulated to maintain n6 fatty acids at the same concentration. Increases in the n3 lipid source, herring oil, were matched by corresponding decreases in triolein.

Table 1.

Composition of experimental diets

	Diets	
	I - V	VI - X
	%	%
Soy protein	14.0	14.0
Wheat	15.0	15.0
Dextrose	10.0	10.0
Herring meal	23.6	23.6
Feather meal	5.0	5.0
Vitamin mix[1]	1.0	1.0
Mineral mix[2]	5.0	5.0
60% Choline chloride	0.6	0.6
Ascorbic acid	0.2	0.2
Lignosulfonate	2.0	2.0
α-Cellulose	12.1	6.1
Lipid[3]	11.4	17.5

[1] The vitamin premix supplied the following levels of nutrient per kg of dry diet: vitamin A acetate 7900 IU, cholecalciferol 790 IU, DL-alpha-tocopheryl acetate 790 IU, menadione 25 mg, D-calcium pantothenate 160 mg, pyridoxine HCl 40 mg, riboflavin 80 mg, niacin 315 mg, folic acid 20 mg, thiamin HCl 50 mg/kg, biotin 4 mg, cyanocobalamin 0.1 mg, inositol 1580.

[2] The mineral premix supplied the following levels of nutrient per kg of dry diet: calcium (as $Ca(H_2PO_4)2H_2O$, $CaHPO_4$, $Ca_{10}(OH)_2(PO_4)_6$) 3370 mg, phosphorus (as $Ca(H_2PO_4)2H_2O$, $CaHPO_4$, $Ca_{19}(OH)_2(PO_4)_6$, Na_2HPO_4) 18283 mg, magnesium (as $MgSO_4*7H_2O$) 300 mg, manganese (as $MnSO_/*5H_2O$) 15 mg, zinc (as $ZnSO_4*7H_2O$) 40 mg, iron (as $FeSO_4*7H_2O$) 70 mg, copper (as Cu SO_4*5H_2O) 1.5 mg, cobalt (as $CoCl*6H_2O$) 2.5 mcg, potassium (as K_2SO_4 and KIO_3) 710 mg, sodium (as NaCl) 790 mg, fluorine (as NaF) 4 mg, selenium (as ($Na_2SeO_3*5H_2O$) 0.1 mg, iodine (as KIO_3) 4 mg.

[3] See table 2 for type of lipid used.

Table 2

Lipid content of the experimental diets

	Lipid Source		
	Herring oil[1]	Triolein[2]	Corn oil
I	1.0	8.9	1.5
II	2.4	7.5	1.5
III	3.8	6.1	1.5
IV	5.2	4.7	1.5
V	6.6	3.3	1.5
VI	1.0	15.0	1.5
VII	2.4	13.6	1.5
VIII	3.8	12.2	1.5
IX	5.2	10.8	1.5
X	6.6	9.4	1.5

[1] The herring oil was generously supplied by Moore-Clark Co.(Canada) Inc., Vancouver, British Columbia, Canada

[2] Obtained from United States Biochemical Corporation, Cleveland, Ohio, USA

Table 3

Determined total lipid and dry matter composition in the diets.

Diet	Dry Matter %	Crude Lipid % DMB
I	77.88	17.33
II	74.02	17.51
III	78.04	17.85
IV	71.19	17.91
V	81.04	18.39
VI	81.66	24.15
VII	83.26	25.12
VIII	79.24	25.06
IX	82.95	24.51
X	81.33	25.23

Table 4

Determined fatty acid composition of the diets.

Concentrations are expressed as percentages of the diet on a dry matter basis.

	(% DMB) Classes of fatty acids					
Diet	sat*	monounsat*	18:1	n3	n3 HUFA*	n6
	%	%	%	%	%	%
I	2.48	10.21	7.60	1.11	0.93	2.04
II	2.74	9.61	6.94	1.64	1.34	1.95
III	3.18	9.27	7.19	2.07	1.76	2.03
IV	3.38	7.93	5.70	2.72	2.34	1.89
V	3.59	7.43	4.90	2.89	2.47	1.84
VI	2.99	15.06	12.15	1.38	1.10	2.39
VII	3.49	15.47	12.52	1.94	1.70	2.52
VIII	3.93	14.10	11.31	2.21	1.89	2.33
IX	4.00	13.55	10.65	2.74	2.35	2.33
X	4.24	13.05	10.07	3.29	2.88	2.28

* sat = total of all saturated fatty acids
monounsat = total of all monounsaturated fatty acids
n3 HUFA = total of all n3 fatty acids > C18

Table 5

Determined fatty acid composition of the diets.

Concentrations are expressed as percentages of the dietary lipid.

	(% of lipid) Classes of fatty acids					
Diet	sat*	monounsat*	18:1	n3	n3 HUFA*	n6
	%	%	%	%	%	%
I	14.29	58.90	43.88	6.41	5.38	11.78
II	15.63	54.86	39.61	9.38	7.68	11.16
III	17.80	51.91	40.29	11.57	9.86	11.38
IV	18.88	44.28	31.85	15.19	13.06	10.58
V	19.51	40.41	26.62	15.71	13.43	10.02
VI	12.37	62.36	50.30	5.71	4.54	9.91
VII	13.90	61.58	49.85	7.73	6.76	10.04
VIII	15.67	56.27	45.15	8.83	7.53	9.30
IX	16.30	55.29	43.45	11.17	9.60	9.51
X	16.80	51.73	39.91	13.03	11.40	9.04

* sat = total of all saturated fatty acids
monounsat = total of all monounsaturated fatty acids
n3 HUFA = total of all n3 fatty acids > C18

The diets were mixed and cold-pelleted in the Animal Science facilities in the MacMillan building and South campus, UBC. The pelleted diet possessed the complete lipid content prior to pelleting (high lipid diets often require part of the lipid to be added after pelleting). Water was added to the dry mixture before being processed through the pellet mill. The diets were stored in a chest freezer at -19° C.

Experimental animals and their environment

Juvenile coho salmon (Oncorhynchus kisutch) from three B.C. strains were unselectively distributed into 30 150 l. tanks in the Aquaculture centre at South campus, UBC using a completely random block design. Strain identity was not retained. Twenty tanks were situated in one room and the remaining ten were in an similar room across the hall. Each tank contained 60 x 9.5 g. fish. Each tank was supplied with dechlorinated aspirated Vancouver city water at the rate of 2 l./min. Figures 1 and 2 portray the water temperature changes over the total experimental period and the growth trial, respectively. The water temperature at the beginning of the experiment was 11.5° C. and fell to 3.5° C. during the winter months.

In May a heat exchanger was installed which provided heated water which remained at a stable temperature ± 1° C. The water temperature was monitored daily. The dissolved O_2 in the water ranged from 9.2 to 11.8 ppm during the experiment. Throughout the study a constant 12 hr. photoperiod was provided by incandescent lighting. The experiment began in October, 1988 and ended in mid July, 1989. The growth trial was postponed until the spring of 1989 because of the low water temperatures during the winter months.

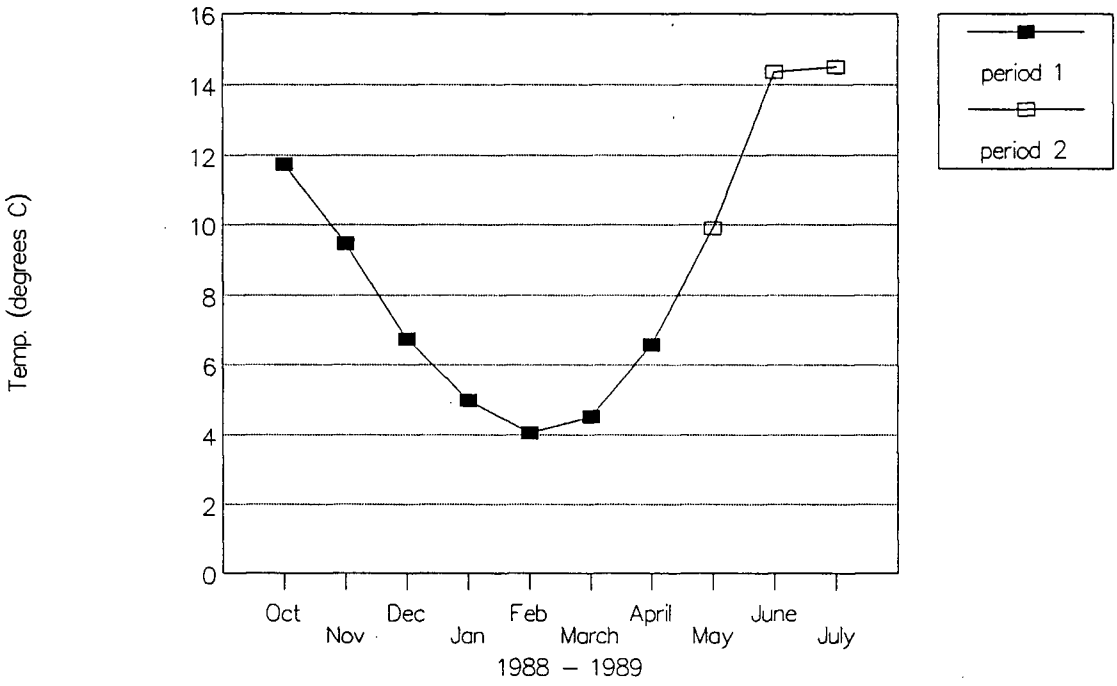

Figure 1. Water temperature for periods 1 and 2, inclusive.

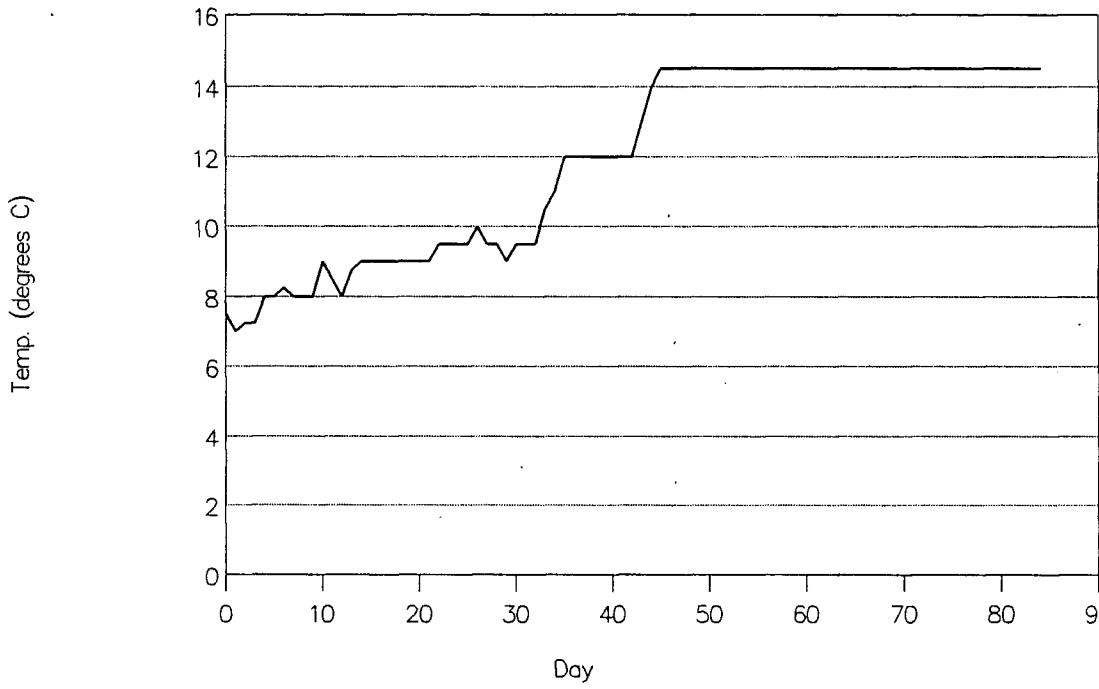

Figure 2. Water temperature during period 2.

The time period (Periods 1 and 2)

The complete term of the experiment was separated into two periods. Period 1 began in October, 1988 and lasted 27 weeks until the water warmed up and the growth trial (period 2) began in April, 1989. Period 2 was 12 weeks long.

Feeding and the monitoring of growth and mortality

The fish were hand-fed in excess of satiation twice daily at the same times each day (0900 and 1500 hrs). The liberal feeding practice was implemented in an attempt to compensate for the poor response to the feed. The amount fed was recorded at each feeding. Mortality was recorded daily. Each fish that died was examined and weighed.

The initial response to the diet was poor. At the beginning of the experiment most of the fish were unresponsive to the diet and the method in which it was distributed. The fish began to respond to the diet after 6 weeks of feeding. However, water temperature began to severely drop at that time. The initial unexpected appetite failure caused the fish to lose weight as they catabolized body stores for energy. At the lower

temperature the fish maintained that lower body weight and did not grow over the winter months. When the water temperature finally began to rise during period 2 many of the fish did not survive. These fish were anorexic and lethargic. The increased water temperature led to an increase in metabolic rate. Growth would have occurred had the fish been eating. However, in the absence of nutrient intake these animals were forced to rely on body stores which were already depleted.

Feed intake data from both experimental periods were deemed as unreliable because of the large number of unresponsive fish. This is associated with the fact that the amount fed was based on a mean fish weight basis. The large number of fish that were not eating resulted in substantial overfeeding. It can be said that those fish which were eating did so to satiation. This, however, can not be quantified.

For the individual weighings the fish were anaesthesized in a 100 ppm methane tricainesulphonate (MS-222), 100 ppm sodium bicarbonate solution. The fish were individually weighed. This procedure was used at the beginning and end of period 1 and 2. Throughout the complete term of the experiment each tank of fish were weighed as a group every three weeks. For the group

weighings the fish were mildly sedated with 2-phenoxyethanol (50 ppm), counted and group-weighed. The group weights were used to monitor the progress of the experiment. They were not, however, used for analysis.

The experiment followed a complete randomized block design. The environments in aquarium rooms 4 and 5 were initially thought to be very similar. However, room 5 was shared with another group of researchers. The block of tanks in room 5 had to be removed from the experiment when the substantially higher traffic in that room was deemed responsible for the poor response from the fish. The mean fish body weight was used in comparisons of growth because the fish were not identified individually.

Sampling procedures

Period 1

An unselected sample of five fish per tank was collected near the end of period 1 while the water temperature was low (5^{o} C). These fish are referred to as the "period 1" specimens. These fish had been on experimental diets for a period of 27 weeks. The fish were anaesthesized, killed and then stored at -20^{o} C. for four months before they were analyzed for lipid content and fatty acid composition. Lipid and fatty acid analyses

were performed on pooled samples consisting of one fish from each of the three blocks per treatment. These fish were selected for analysis on the basis of body condition and evidence of ingestive activity.

Period 2

At the termination of the 12-week feeding trial the fish were individually weighed. Those fish not being used for a saltwater challenge described below were killed. These fish were stored for two weeks at -20° C. before being analyzed for dry matter and lipid. Lipid and fatty acid analyses were performed on four fish per tank. Fish from the first two blocks were analyzed. The blocks were not pooled in this case (see above).

Analysis of body lipids

Total lipid was extracted from ground whole fish according to Bligh and Dyer (1959) with modifications by Christie (1973) and the resulting lipid separated into neutral and polar fractions with the use of two Waters-Millipore Sep-Paks (Waters Associates, Milford, Massachussetts) connected in series. Fatty acid methyl esters were produced by base-catalyzed fatty acid methyl esterification (Christie, 1982). Fatty acid methyl

esters were analyzed using a Varian 3700 gas chromatograph equipped with a Supelcowax-10 column (30m,.32mm ID) (Supelco Canada Ltd., Oakville, Ont.). The injection and detector port temperatures were both 220°C. Oven temperatures ranged from 170° to 230°C, increasing at a rate of 2°C/min after an initial 6 minutes at 170°C.

The fatty acids were identified by reference to Supelco PUFA-1 and Rapeseed oil standards (Supelco Canada Ltd., Oakville, Ont.). The fatty acid concentrations were calculated as either a percentage of their respective lipid fraction or as a percentage of the non-lipid dry body weight of the fish. The latter method of calculation was used to remove variation in body lipid content within and between treatments.

In the neutral fraction, the percent of each class of fatty acid (NFA) was calculated as follows:

$$NFA = (F_n \times N)/(D + P) \times 100$$

where NFA = percent of that fatty acid class in the neutral lipid as a function of the dry nonlipid structural body weight

F_n = concentration of a fatty acid class in the neutral lipid

N = quantity of neutral lipid in the body (g)

D = dry nonlipid body weight (g)

P = quantity of polar lipid in the body (g)

The polar lipid was added to the denominator in the standardization of the neutral lipid because the polar

lipid is believed to be structural.

In the case of body polar lipid, the percent of each class of fatty acid (PFA) was calculated as follows:

$$PFA = (F_p \times P)/D \times 100$$

where PFA = percent of that fatty acid class in the polar lipid as a function of the dry nonlipid body weight
F_p = concentration of a fatty acid class in the polar lipid
P = quantity of polar lipid in the body (g)
D = dry nonlipid body weight

Both NFA and PFA are expressed as percent (%).

Saltwater challenge

Five fish from each tank, using all three blocks, were revived after anaesthesia and used for a saltwater challenge. The saltwater challenge was based on the procedure of Blackburn and Clark (1987). The fish were placed into one of ten chambers used for the challenge. The fish from all three blocks of a treatment were placed into the same chamber. Caudal fin clips were used to differentiate the different blocks. The fish remained in the blackened chambers for 24 hours. The chambers were provided with full strength artificial saltwater (28 parts per thousand). The water was recirculated, aerated (9 ppm O_2) and cooled (16^{o} C.).

At the end of the 24 hour saltwater challenge the

fish were anaesthesized for the collection of blood samples. Blood was collected in heparinized capillary tubes from two fish for each treatment and each block. The blood was centrifuged and the plasma was refrigerated for 24 hours before being analyzed for plasma sodium levels using a Corning 410 flame photometer.

Muscle samples were taken from two unselectively chosen fish in freshwater and from two fish immediately following the 24 hour saltwater challenge. A section of skinless, boneless muscle was extracted from killed fish from an area posterior to the dorsal fin, anterior to the adipose fin on the dorsal side of the lateral line on one side of the spinal column. Muscle samples were stored at -19° C. for 48 hours before determining dry matter concentration.

Statistical analysis

A comparison of regressions was used for the statistical analysis of the body fatty acid composition. The dependent variable in the regressions was diet fatty acid content. It was expressed as either percent of the dry diet (% DMB) or percent of the dietary lipid (% lipid). Seven classes of fatty acids were used as

independent variables. They were total saturated, total monounsaturated, total 18:1, total n3, n3 highly unsaturated, total n6, and n6 highly unsaturated fatty acids. The dependent variable was one of the seven classes of fatty acids found in the polar or neutral fraction of the body lipid. It was calculated as grams of fatty acid (in either polar or neutral lipid) divided by the dry nonlipid body weight.

The data on fatty acid composition were analysed according to Snedecor and Cochran (1967). First, the residual mean squares of the individual regressions for the body lipid fatty acid composition of either the high or low dietary lipid group as a function of a dietary fatty acid class was compared by a two-tailed F-test to determine whether there was homogeneity of residual variances. If there were homogeneous residual variances then the two slopes were compared using another F-test. If the slopes were not significantly different then an F-test of the difference between adjusted means was performed to determine whether the elevations of two regression differed significantly. If the elevations were not found to be significantly different then pooling of the data from the two dietary groups (diets I-V and VI-X) was justified. T-tests were performed on all regressions

to determine whether they were significantly different from a slope of zero. The regressions with significant slope were then compared on the basis of their r^2 value.

Analysis of variance was performed on the growth rate data. The mortality data were analyzed using a Chi squared test and by the regression analysis mentioned above. Analysis of variance was performed on the muscle dry matter data from the saltwater challenge.

Results

Body Fatty acid composition

The results from the fatty acid analysis are reported separately for the two sampling periods. These periods, "period 1" and "period 2", have been defined in the materials and methods section of this document. The major fatty acid groups in the polar lipid of the period 1 fish is reported in table 7 as a percent of the polar lipid. In table 8 the polar fatty acid groups are reported as a percentage of the dry non-lipid body weight. The latter method of quantifying the fatty acids was used in an attempt to compensate for variation in the ratio of polar lipid to total lipid as well as the amounts of total lipid among and within treatments thereby giving an accurate assessment of the relationship between that lipid fraction and structural body mass. Table 6 lists the polar, neutral and total lipid content of the fish. The neutral lipid fatty acid composition is expressed as a percent of the neutral lipid in table 9. Table 10 reports the major fatty acid groups in the neutral lipid as percentages of the dry nonlipid body weight plus the amount of polar lipid. The data for the period 2 fish are listed in

tables 12 through 16. These tables correspond with tables 6 - 10 for the period 1 fish.

A. The fatty acid composition of fish sampled in Period 1

Table 6

Dry matter and lipid composition of fish at the end of period 1.

Concentrations of polar and neutral body lipid are expressed as percentages of the total lipid or of the non-lipid dry structural body weight.

Diet	% DM*	% lipid DMB	polar/ total lipid (%)	neutral/ total lipid (%)	polar/ nonlipid dry b.wt (%)	neutral/ nonlipid dry b.wt+ polar (%)
I	21.91	25.35	23.79	76.21	8.81	25.93
II	23.16	24.35	16.67	83.33	5.37	25.46
III	23.31	20.27	24.56	75.44	6.24	18.03
IV	21.77	21.87	20.35	79.65	5.69	21.09
V	23.84	24.20	31.78	68.22	10.15	19.78
VI	21.28	29.98	24.22	75.78	10.37	29.40
VII	21.18	33.52	23.73	76.27	11.97	34.35
VIII	24.88	30.71	22.61	77.39	10.02	31.17
IX	20.16	23.11	31.53	68.47	9.48	18.80
X	26.97	26.40	22.22	77.78	7.97	25.84

*DM = dry matter

Table 7

Fatty acid composition of polar lipid extracted from fish at the end of period 1.

Concentrations of fatty acids are expressed as percentages of the polar lipid.

Diet	Classes of fatty acids						
	sat*	monounsat*	18:1	n3	n3 HUFA*	n6	n6 HUFA*
	%	%	%	%	%	%	%
I	21.93	34.67	18.30	34.84	30.88	3.60	ND**
II	17.26	43.94	16.42	28.23	28.23	2.81	ND
III	17.59	28.57	15.21	25.78	25.78	3.87	ND
IV	22.91	25.97	13.95	26.70	26.70	3.06	ND
V	22.12	36.13	15.74	30.62	29.12	3.01	ND
VI	22.85	47.23	27.04	24.96	23.74	4.95	ND
VII	20.68	44.91	25.90	29.53	28.14	4.33	ND
VIII	23.25	47.43	26.16	24.08	22.70	5.25	ND
IX	28.13	37.77	22.03	30.28	30.28	3.60	ND
X	22.22	38.12	20.65	30.68	29.33	4.43	ND

* sat = total of all saturated fatty acids
monounsat = total of all monounsaturated fatty acids
n3 HUFA = total of all n3 fatty acids > C18
n6 HUFA = total of all n6 fatty acids > C18
** ND = not detected

Table 8

Fatty acid composition of polar lipid extracted from fish at the end of period 1.

Concentrations of fatty acids are expressed as percentages of the non-lipid dry body weight.

Diet	Classes of fatty acids						
	sat*	monounsat*	18:1	n3	n3 HUFA*	n6	n6 HUFA*
	%	%	%	%	%	%	%
I	1.93	3.05	1.61	3.07	2.72	0.32	ND
II	0.93	2.36	0.88	1.51	1.51	0.15	ND
III	1.10	1.78	0.95	1.61	1.61	0.24	ND
IV	1.30	1.48	0.79	1.52	1.52	0.17	ND
V	2.24	3.67	1.60	3.11	2.96	0.31	ND
VI	2.37	4.90	2.80	2.59	2.46	0.51	ND
VII	2.47	5.37	3.10	3.53	3.37	0.52	ND
VIII	2.33	4.75	2.62	2.41	2.27	0.53	ND
IX	2.67	3.58	2.09	2.87	2.87	0.34	ND
X	1.77	3.04	1.65	2.45	2.34	0.35	ND

* sat = total of all saturated fatty acids
monounsat = total of all monounsaturated fatty acids
n3 HUFA = total of all n3 fatty acids > C18
n6 HUFA = total of all n6 fatty acids > C18
** ND = not detected

Table 9

Fatty acid composition of neutral lipid extracted from fish at the end of period 1.

Concentrations of fatty acids are expressed as percentages of the neutral lipid.

Diet	Classes of fatty acids						
	sat*	monounsat*	18:1	n3	n3 HUFA*	n6	n6 HUFA*
	%	%	%	%	%	%	%
I	17.14	53.03	28.02	14.52	12.05	7.25	0.78
II	16.69	54.42	23.85	16.09	13.71	5.31	1.10
III	16.76	55.00	24.23	14.30	11.73	5.86	0.74
IV	15.34	46.61	18.90	15.29	12.75	5.80	2.17
V	15.98	53.15	22.07	16.26	14.04	6.35	1.11
VI	15.49	56.67	30.75	11.25	9.46	6.65	1.11
VII	16.10	57.65	32.21	13.67	11.47	6.94	0.66
VIII	16.76	58.53	34.19	13.31	11.35	7.25	0.33
IX	17.10	59.70	24.99	15.10	12.91	4.89	0.36
X	17.20	56.28	28.77	15.80	13.33	6.96	0.61

* sat = total of all saturated fatty acids
  monounsat = total of all monounsaturated fatty acids
  n3 HUFA = total of all n3 fatty acids > C18
  n6 HUFA = total of all n6 fatty acids > C18
** ND = not detected

Table 10

Fatty acid composition of neutral lipid extracted from fish at the end of period 1.

Concentrations of fatty acids are expressed as percentages of the non-lipid dry structural body weight.

	Classes of fatty acids						
Diet	sat*	monounsat*	18:1	n3	n3 HUFA*	n6	n6 HUFA*
	%	%	%	%	%	%	%
I	4.44	13.75	7.27	3.77	3.12	1.88	0.20
II	4.25	13.85	6.10	4.10	3.49	1.35	0.28
III	3.02	9.92	4.37	2.58	2.11	1.06	0.13
IV	3.23	9.83	3.99	3.22	2.69	1.22	0.46
V	3.16	10.51	4.36	3.22	2.78	1.26	0.22
VI	4.55	16.66	9.04	3.31	2.78	1.96	0.32
VII	5.53	19.80	11.06	4.70	3.94	2.38	0.23
VIII	5.22	18.25	10.66	4.15	3.54	2.26	0.10
IX	3.22	11.23	4.70	2.84	2.43	0.92	0.07
X	4.44	14.54	7.43	4.08	3.44	1.80	0.16

* sat = total of all saturated fatty acids
monounsat = total of all monounsaturated fatty acids
n3 HUFA = total of all n3 fatty acids > C18
n6 HUFA = total of all n6 fatty acids > C18
** ND = not detected

I. Saturated fatty acids

Pooled regressions were not obtained from the regressions of saturated fatty acids in the body neutral lipid fraction as a function of dietary saturates (% DMB) and of dietary n3 (% DMB) because of significant differences in the elevations of the high and low lipid groups.

The regressions of saturated fatty acids in the body polar lipid as functions of n3 in the dry diet (% DMB) and in the dietary lipid (% lipid) also produced significant differences in the elevations of high and low lipid groups.

There were significant slopes produced in the regressions of saturated fatty acids in the body neutral lipid as a function of saturated fatty acids (% lipid) or dietary n3 (% lipid). These regressions had r^2 values of 0.54 and 0.47 respectively. The regressions are shown in figures 4 and 5, respectively.

The only other significant slope was produced by a regression of saturated fatty acids in the body neutral lipid as a function of dietary saturates (% DMB) in the low lipid group. This regression produced an r^2 value of 0.83. The regression is shown in figure 3.

There also were significant slopes produced by the regressions of neutral body saturated fatty acids as functions of dietary monounsaturates (% DMB and % lipid) and dietary 18:1 (% DMB and % lipid). The r^2 values of these regressions were 0.50, 0.49, 0.51 and 0.50, respectively. These regressions are shown in figures 6 - 9, respectively.

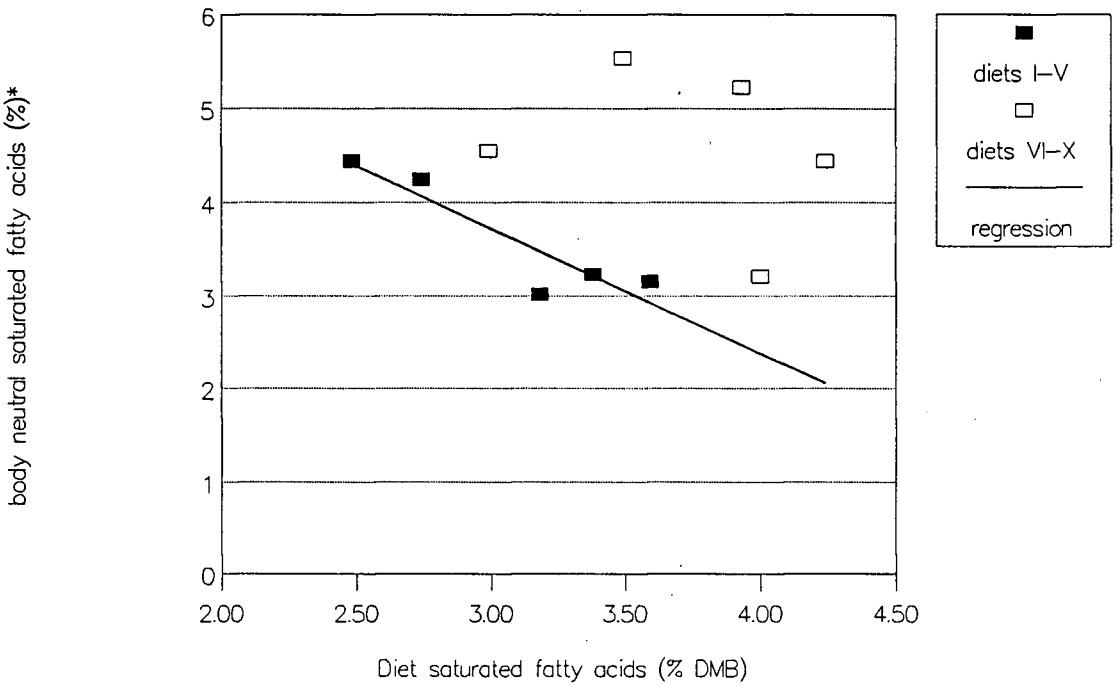

Figure 3. Saturated fatty acid concentration in the body neutral lipid as a function of dietary saturated fatty acid concentration (% DMB) in period 1. The regression includes the low lipid diets (I-V) only.
*body fatty acids expressed as % NFA (see page 56)
$y = -1.34x + 7.74$ $r^2 = 0.83$

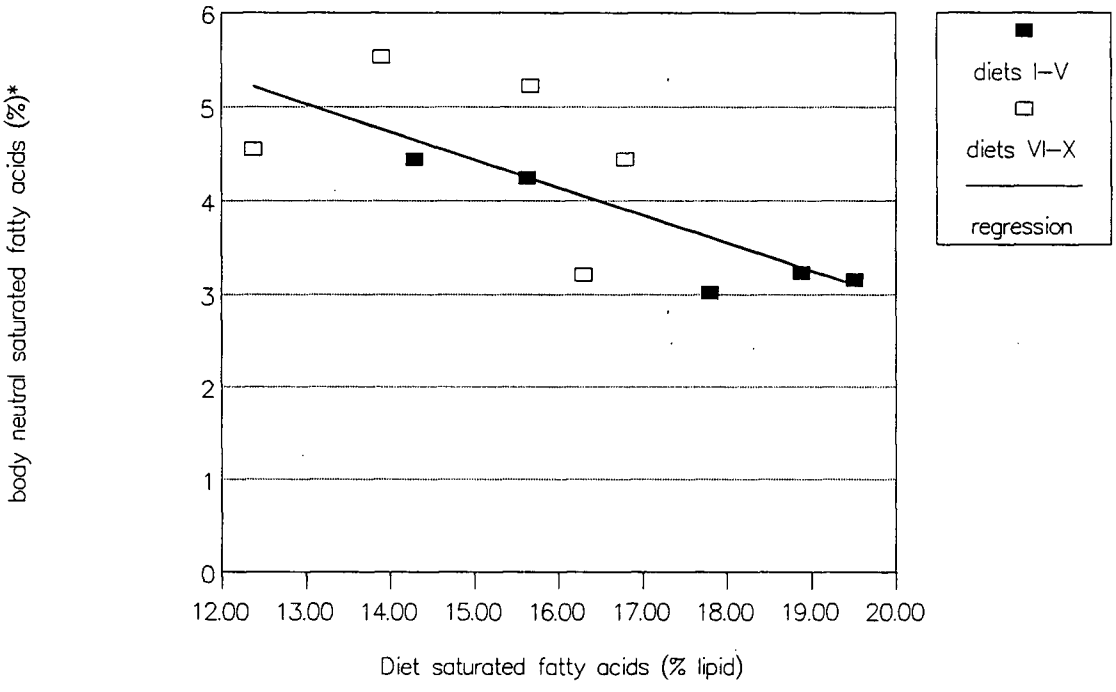

Figure 4. Saturated fatty acid concentration in the body neutral lipid as a function of dietary saturated fatty acid concentration (% lipid) in period 1. The regression involves all diets (I-X).

*body fatty acids expressed as % NFA (see page 56)

$y = -0.30x + 8.88 \quad r^2 = 0.54$

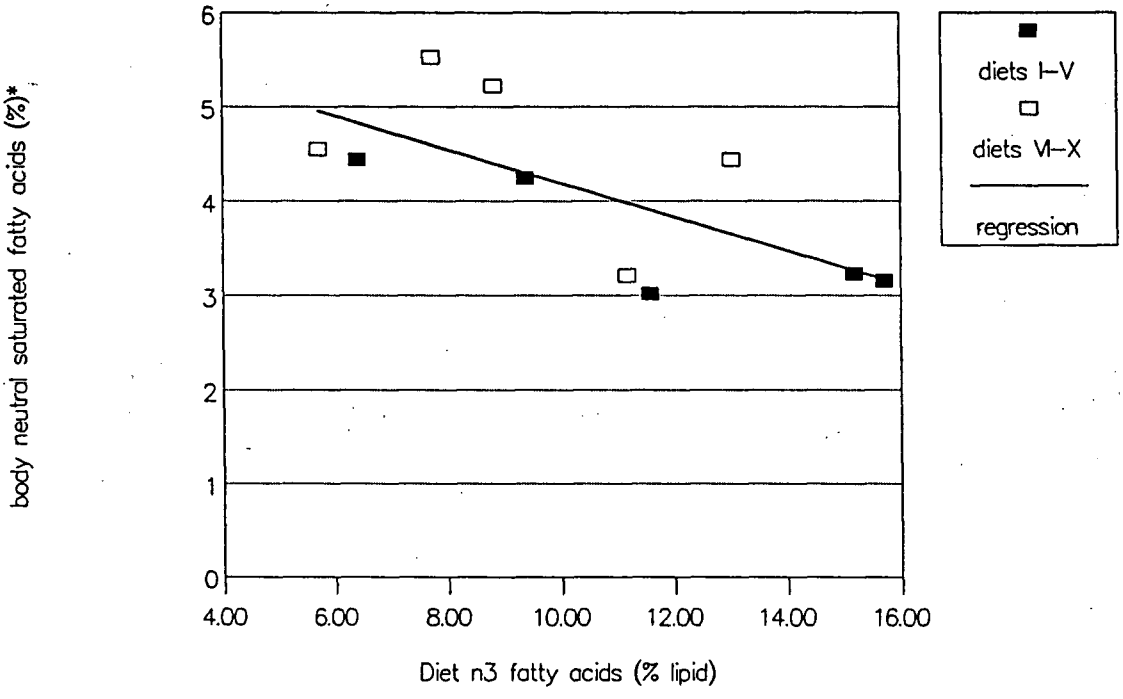

Figure 5. Saturated fatty acid concentration in the body neutral lipid as a function of dietary n3 fatty acid concentration (% lipid) in period 1. The regression involves all diets (I-X).
*body fatty acids expressed as % NFA (see page 56)
$y = 0.18x + 5.98$ $r^2 = 0.47$

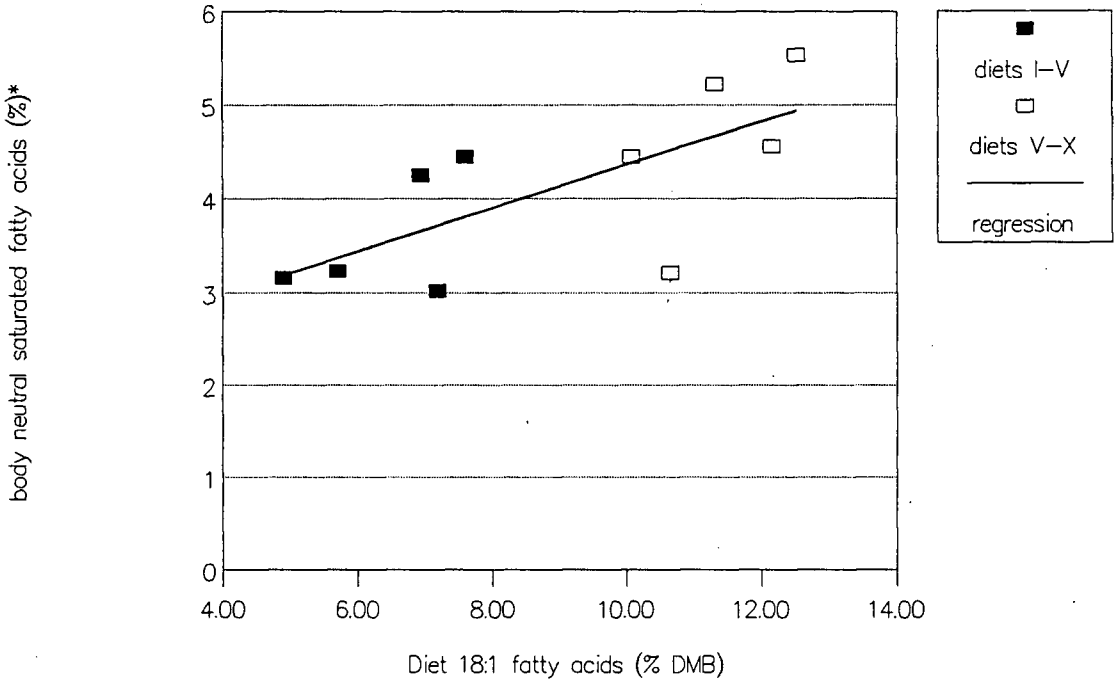

Figure 6. Saturated fatty acid concentration in the body neutral lipid as a function of dietary 18:1 fatty acid concentration (% DMB) in period 1. The regression involves all diets (I-X).
*body fatty acids expressed as % NFA (see page 56)
$y = 0.23x + 2.05$ $r^2 = 0.50$

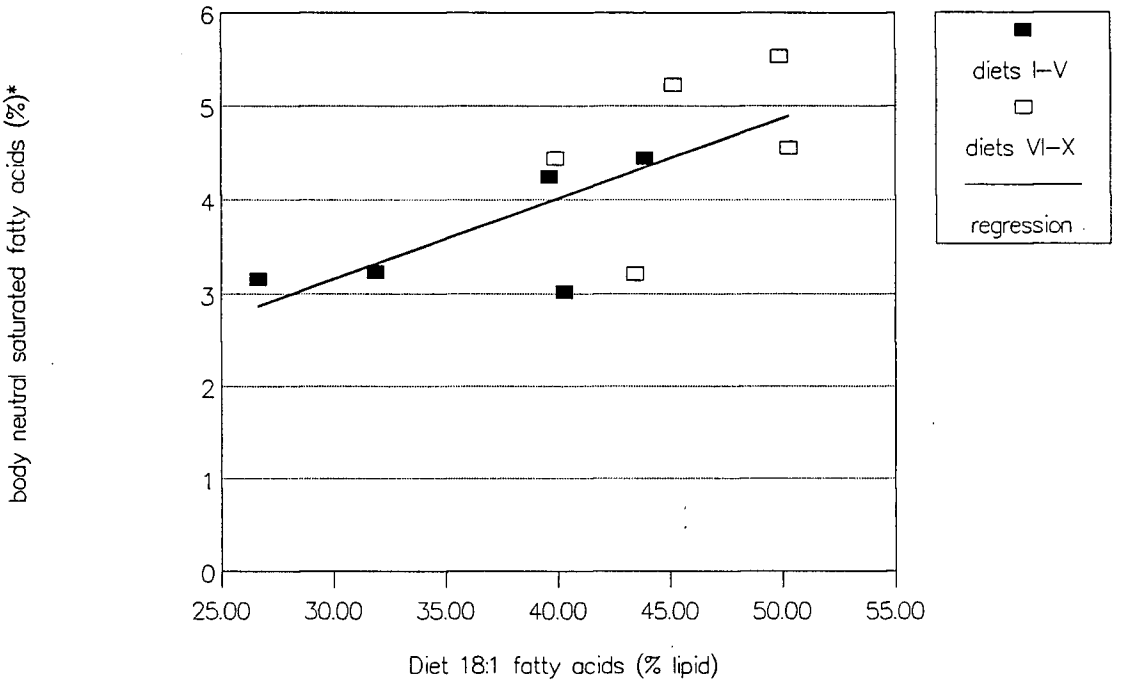

Figure 7. Saturated fatty acid concentration in the body neutral lipid as a function of dietary 18:1 fatty acid concentration (% lipid) in period 1. The regression involves all diets (I-X).
*body fatty acids expressed as % NFA (see page 56)
$y = 0.09x + 0.59$ $r^2 = 0.49$

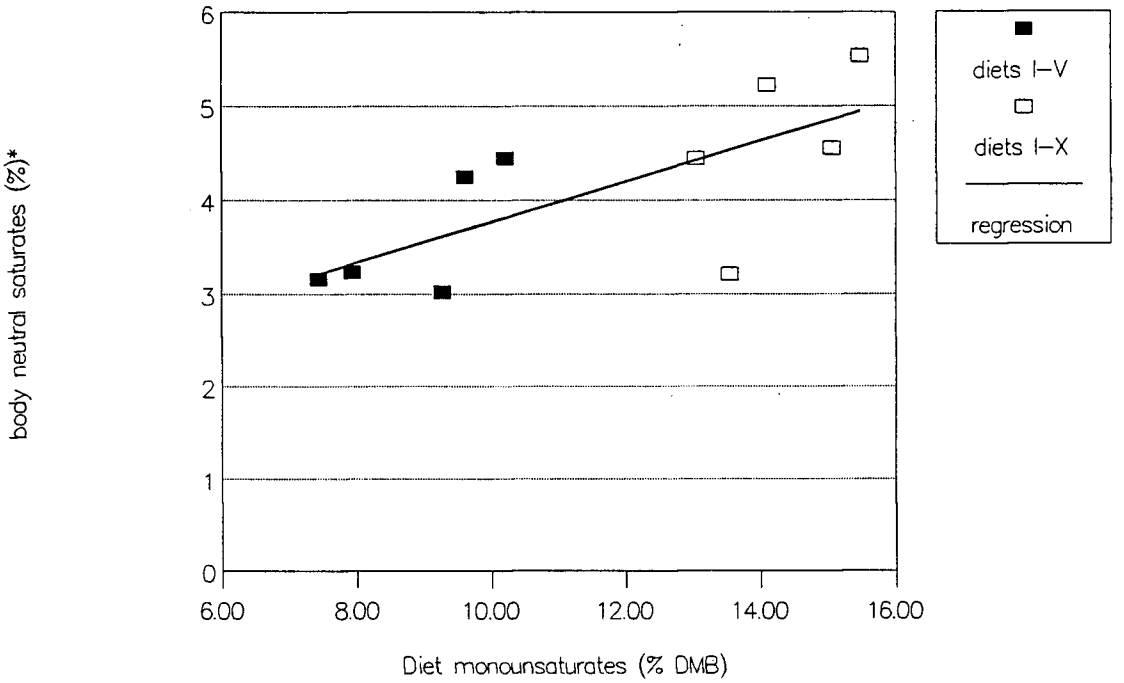

Figure 8. Saturated fatty acid concentration in the body neutral lipid as a function of dietary monounsaturated fatty acid concentration (% DMB) in period 1. The regression involves all diets (I-X).
*body fatty acids expressed as % NFA (see page 56)
$y = 0.22x + 1.61$ $r^2 = 0.51$

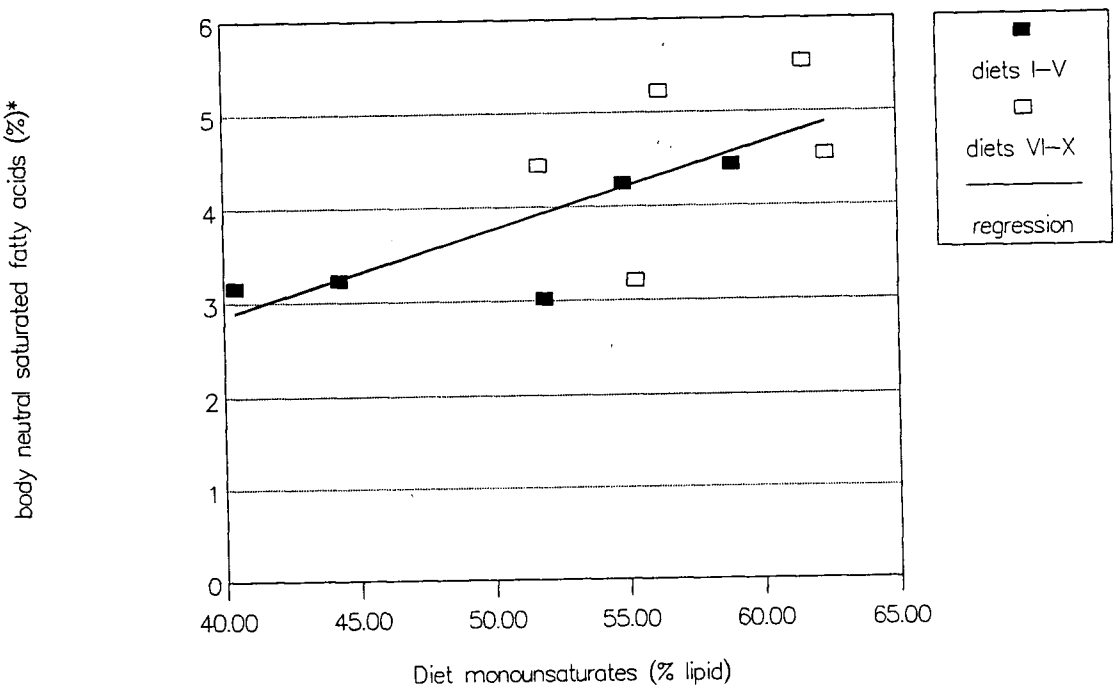

Figure 9. Saturated fatty acid concentration in the body neutral lipid as a function of dietary monounsaturated fatty acid concentration (% lipid) in period 1. The regression involves all diets (I-X).
*body fatty acids expressed as % NFA (see page 56)
$y = 0.09x - 0.77$ $r^2 = 0.50$

II. Monounsaturated fatty acids

Significant differences in elevation between the high and low dietary lipid groups were observed for the regressions of monounsaturated fatty acids as a function of n3 in the dry diet (% DMB). Similar differences in elevations occurred within the regressions of monounsaturated fatty acids in the body polar lipid as functions of dietary n3 (% DMB and % lipid).

There were significant slopes produced in the regressions of monounsaturated fatty acids in the body neutral lipid as functions dietary monounsaturates (% DMB and % lipid) shown in figures 10 and 11, respectively. The slope of the regression of monounsaturates as a function of dietary n3 (% lipid) was also significant (figure 13). The r^2 values of these three regressions were 0.66, 0.54 and 0.49 respectively.

The regression of monounsaturated fatty acid content in the body polar lipid as a function of dietary monounsaturates (% DMB) produced a significant slope. This regression produced an r^2 value of 0.63. The regression is shown in figure 12.

The regressions of monounsaturated fatty acid content in the body polar lipid as functions of dietary n3 (% DMB)

and (% lipid) for the high lipid group produced significant slopes. The r^2 values of these two regressions were 0.79 and 0.81. The regressions are shown in figures 14 and 15, respectively.

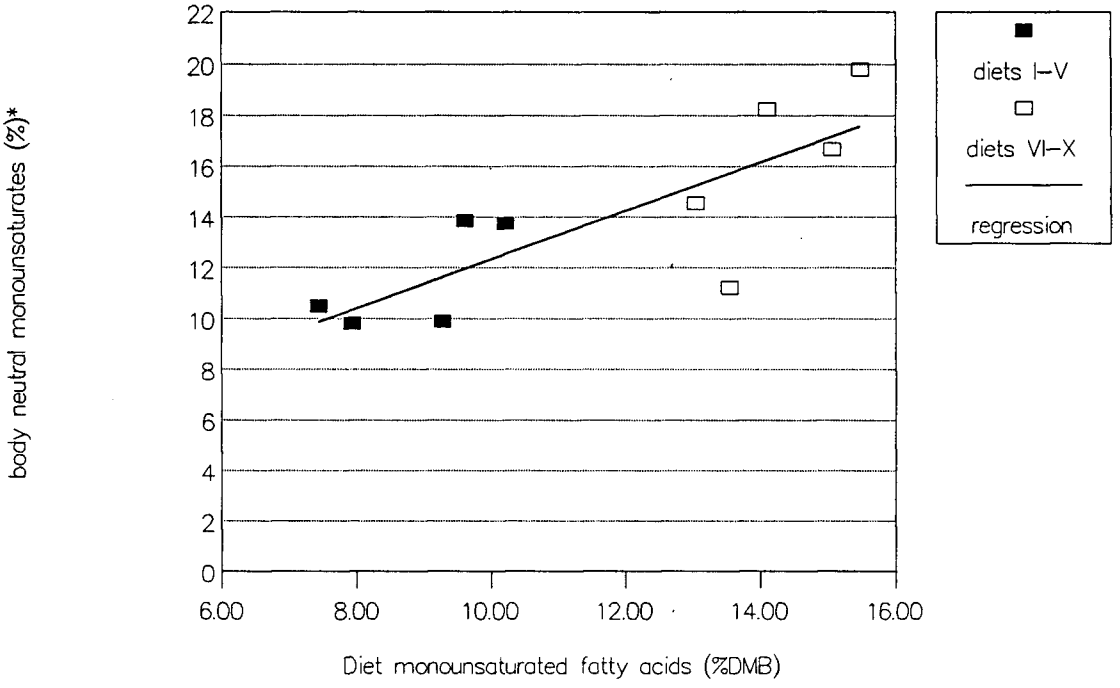

Figure 10. Monounsaturated fatty acid concentration in the body neutral lipid as a function of dietary monounsaturated fatty acid concentration (% DMB) in period 1. The regression involves all diets (I-X).

*body fatty acids expressed as % NFA (see page 56)

$y = 0.95x + 2.79$ $r^2 = 0.66$

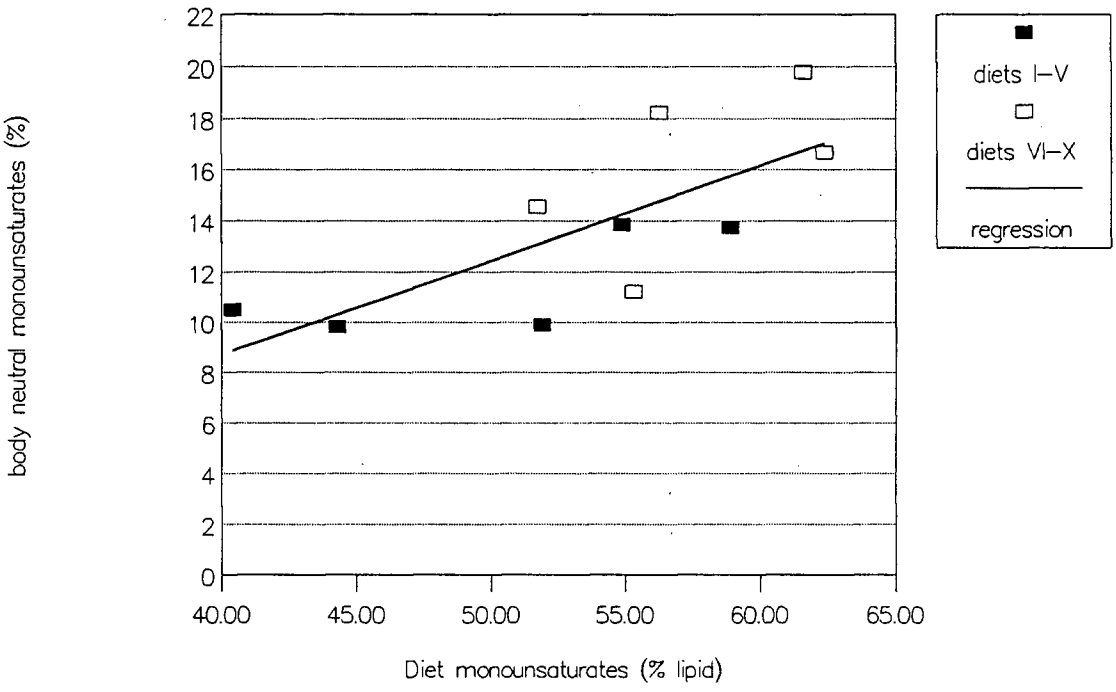

Figure 11. Monounsaturated fatty acid concentration in the body neutral lipid as a function of dietary monounsaturated fatty acid concentration (% lipid) in period 1. The regression involves all diets (I-X).
*body fatty acids expressed as % NFA (see page 56)
$y = 0.37x - 6.10$ $r^2 = 0.54$

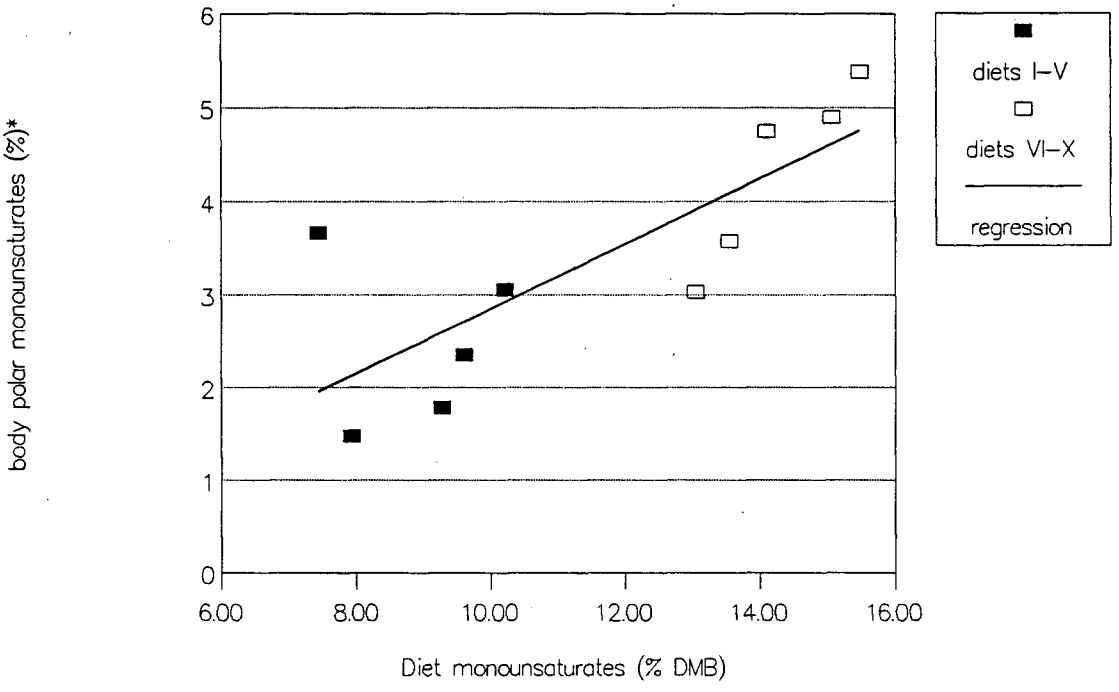

Figure 12. Monounsaturated fatty acid concentration in the body polar lipid as a function of dietary monounsaturated fatty acid concentration (% DMB) in period 1. The regression involves all diets (I-X).
*body fatty acids expressed as % PFA (see page 57)
$y = 0.35x - 0.63$ $r^2 = 0.63$

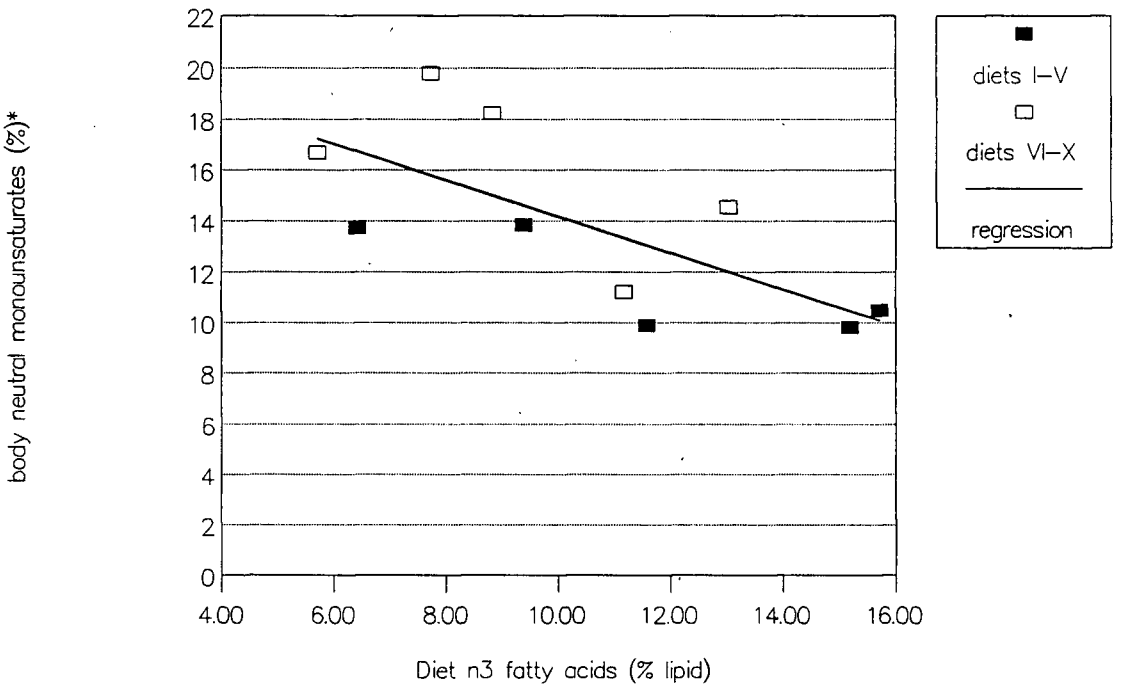

Figure 13. Monounsaturated fatty acid concentration in the body neutral lipid as a function of dietary n3 fatty acid concentration (% lipid) in period 1. The regression involves all diets (I-X).

*body fatty acids expressed as % NFA (see page 56)

$y = -0.71x + 21.30$ $r^2 = 0.49$

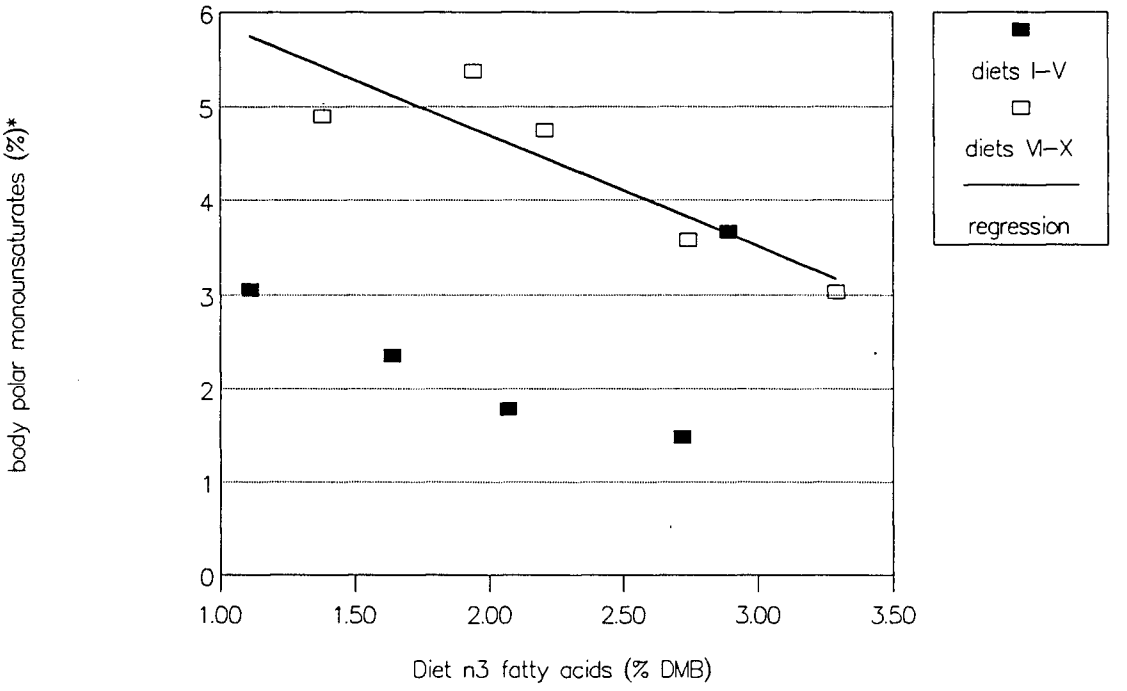

Figure 14. Monounsaturated fatty acid concentration in the body polar lipid as a function of dietary n3 fatty acid concentration (% DMB) in period 1. The regression involves the high lipid diets (VI-X) only.
*body fatty acids expressed as % PFA (see page 57)
$y = -1.18x + 7.06$ $r^2 = 0.79$

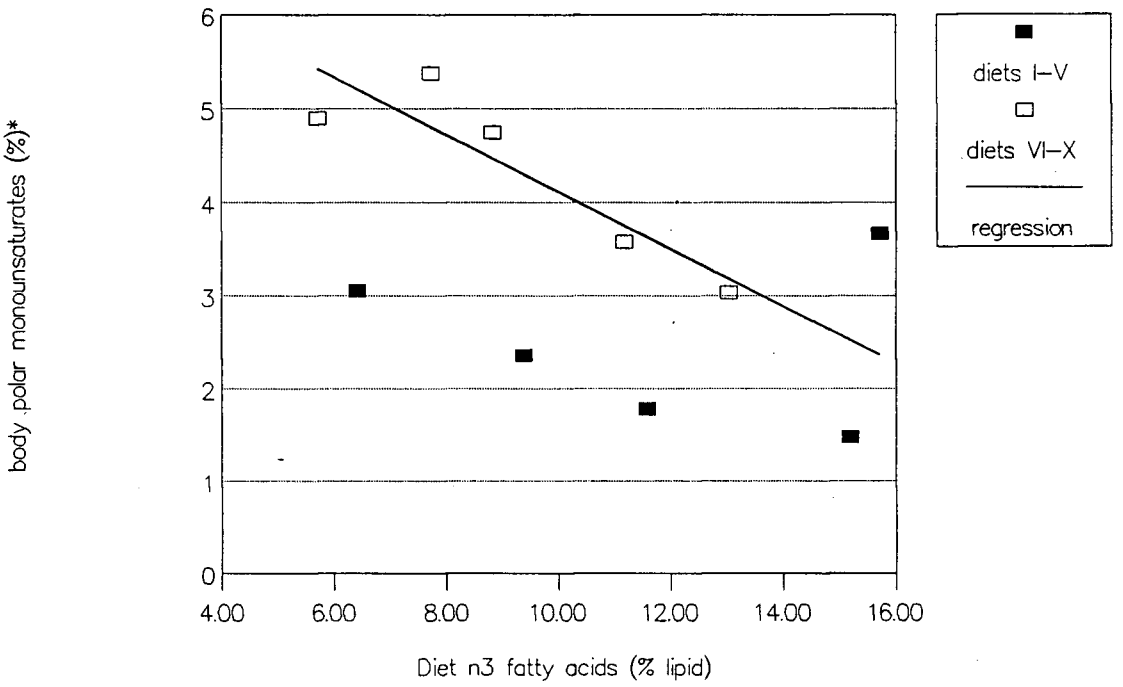

Figure 15. Monounsaturated fatty acid concentration in the body polar lipid as a function of dietary n3 fatty acid concentration (% lipid) in period 1. The regression involves the high lipid diets (VI-X) only.
*body fatty acids expressed as % PFA (see page 57)
$y = -0.31x + 5.57$ $r^2 = 0.81$

III. C18 Monounsaturated fatty acids

In the body neutral lipid fraction a significant difference in elevations between high and low dietary lipid groups occurred when dietary n3 (% DMB) was used as the independent variable.

Significant slopes resulted from regressions of body neutral 18:1 fatty acid content as a function of dietary 18:1 (% DMB or % lipid) and dietary n3 (%lipid). the r^2 values of these regressions were 0.64, 0.57 and 0.50. These regressions are shown in figures 16, 17 and 19, respectively. These values indicated that categorizing the independent variable in terms of the amount in the dry diet was only slightly better than using the percent in the lipid. Dietary 18:1 (% lipid) proved to be only slightly more responsible for variations in the neutral body lipid than was dietary n3 (% lipid).

A significant slope was produced by the regression of 18:1 fatty acid concentration in the body neutral lipid as a function of dietary n3 (% DMB) in the low lipid group. The r^2 value for that regression was 0.85. This regression is shown in figure 18.

The regressions involving 18:1 fatty acids in the body polar lipid were difficult to interpret. The

regressions using dietary n3 (% DMB and % lipid) both produced significant differences in the elevations between high and low dietary lipid groups. Of these, the regression of 18:1 fatty acid concentration in the body polar lipid as a function of dietary n3 (% DMB or % lipid) in the high dietary lipid group produced a significant slopes. The r^2 values of this regression were 0.80 and 0.82. The regressions are shown in figures 20 and 21, respectively.

A statistical difference in the residual variance between the high and low dietary lipid groups occurred when the 18:1 fatty acid content in the body polar lipid was related to dietary 18:1 (% DMB) suggesting lack of homogeneity.

There was a significant difference between the slopes of high and low dietary lipid groups when a regression using dietary 18:1 (% lipid) was created.

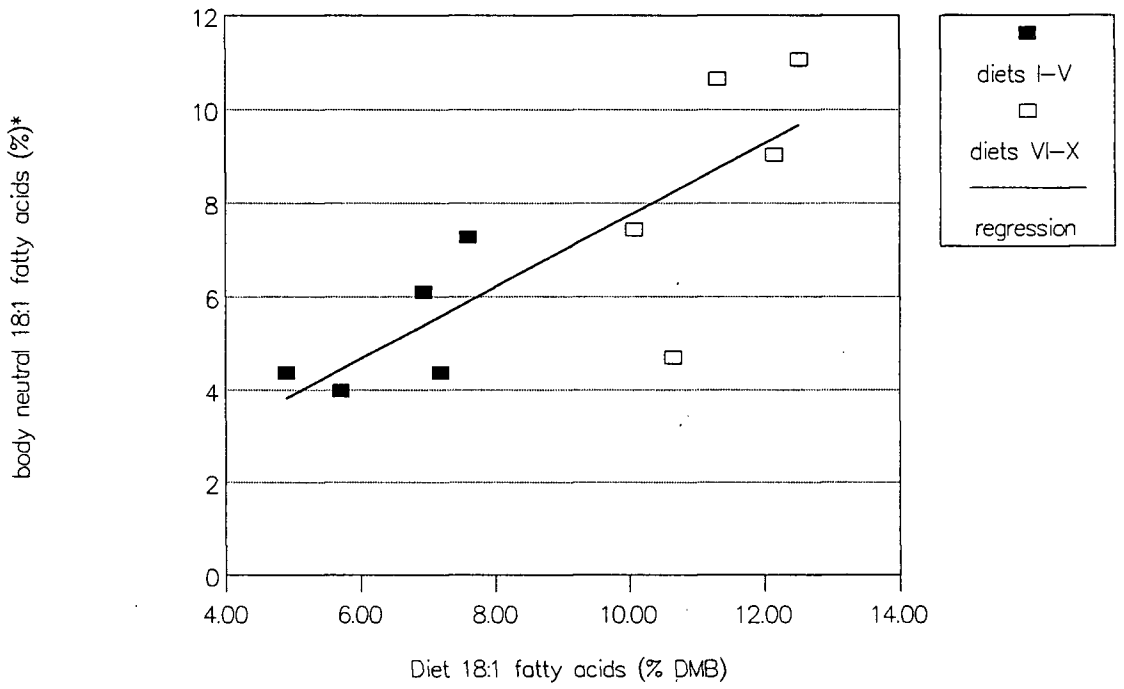

Figure 16. 18:1 fatty acid concentration in the body neutral lipid as a function of dietary 18:1 fatty acid concentration (% DMB) in period 1. The regression involves all diets (I-X).
*body fatty acids expressed as % NFA (see page 56)
$y = 0.77x + 0.05$ $r^2 = 0.64$

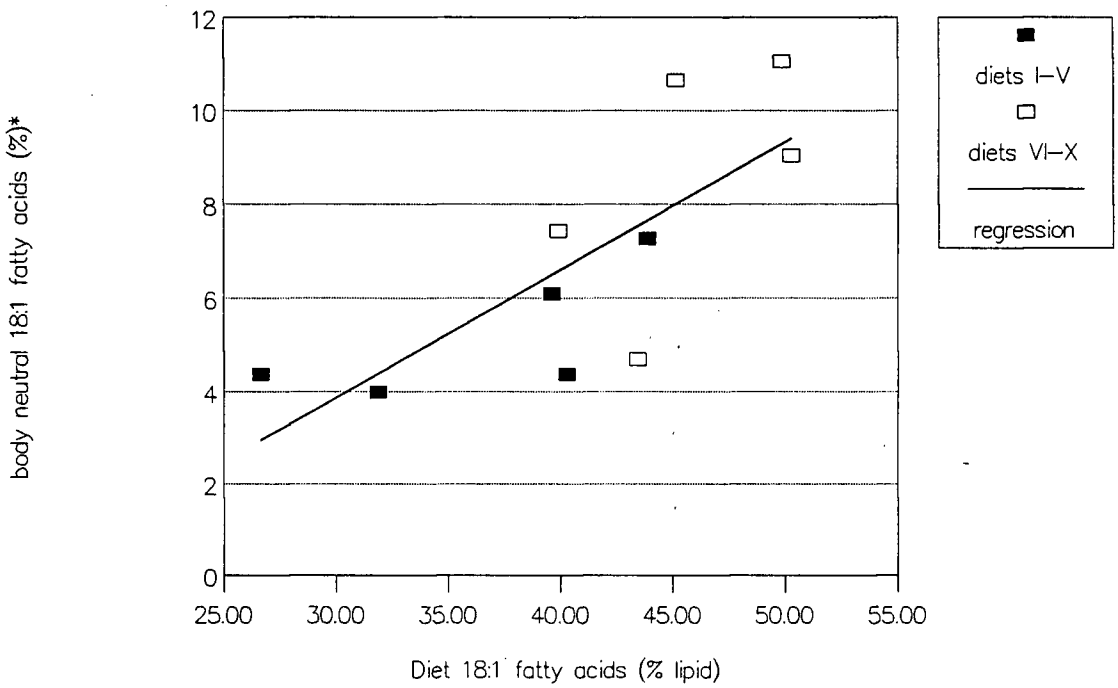

Figure 17. 18:1 fatty acid concentration in the body neutral lipid as a function of dietary 18:1 fatty acid concentration (% lipid) in period 1. The regression involves all diets (I-X).
*body fatty acids expressed as % NFA (see page 56)
$y = 0.27x - 4.29$ $r^2 = 0.57$

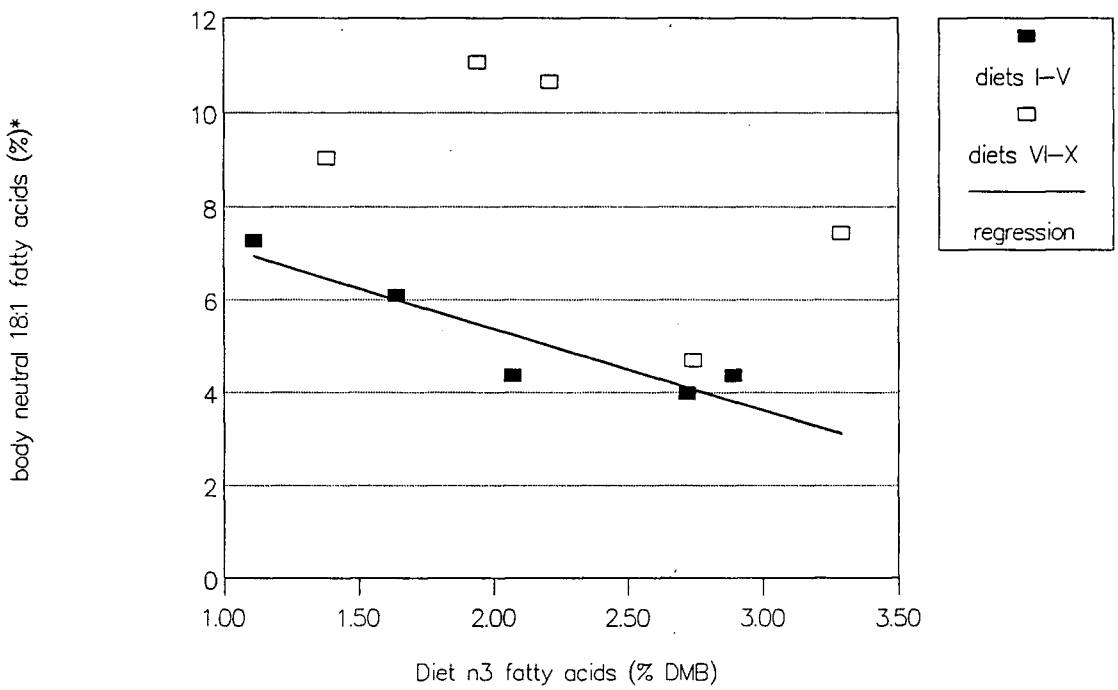

Figure 18. 18:1 fatty acid concentration in the body neutral lipid as a function of dietary n3 fatty acid concentration (% DMB) in period 1. The regression involves the low lipid diets (I-V) only.
*body fatty acids expressed as % NFA (see page 56)
$y = -1.75x + 8.86$ $r^2 = 0.85$

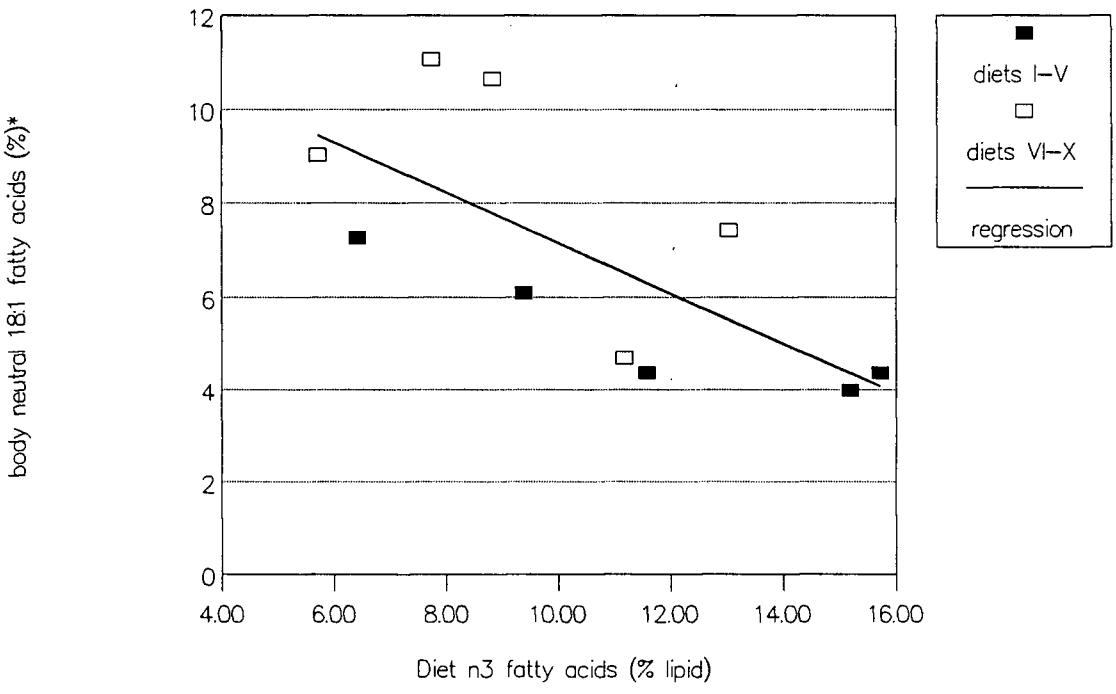

Figure 19. 18:1 fatty acid concentration in the body neutral lipid as a function of dietary n3 fatty acid concentration (% lipid) in period 1. The regression involves all diets (I-X).
*body fatty acids expressed as % NFA (see page 56)
$y = -0.54x + 12.53$ $r^2 = 0.50$

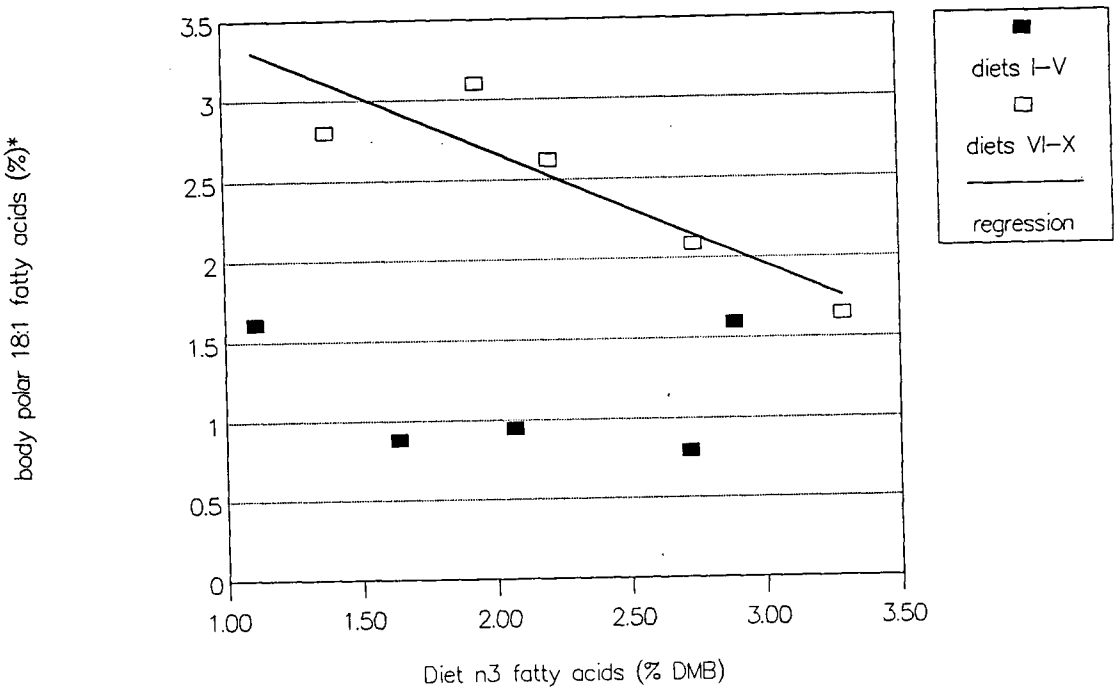

Figure 20. 18:1 fatty acid concentration in the body polar lipid as a function of dietary n3 fatty acid concentration (% DMB) in period 1. The regression involves the high lipid diets (VI-X) only.

*body fatty acids expressed as % PFA (see page 57)

$y = -0.71x + 4.09$ $r^2 = 0.80$

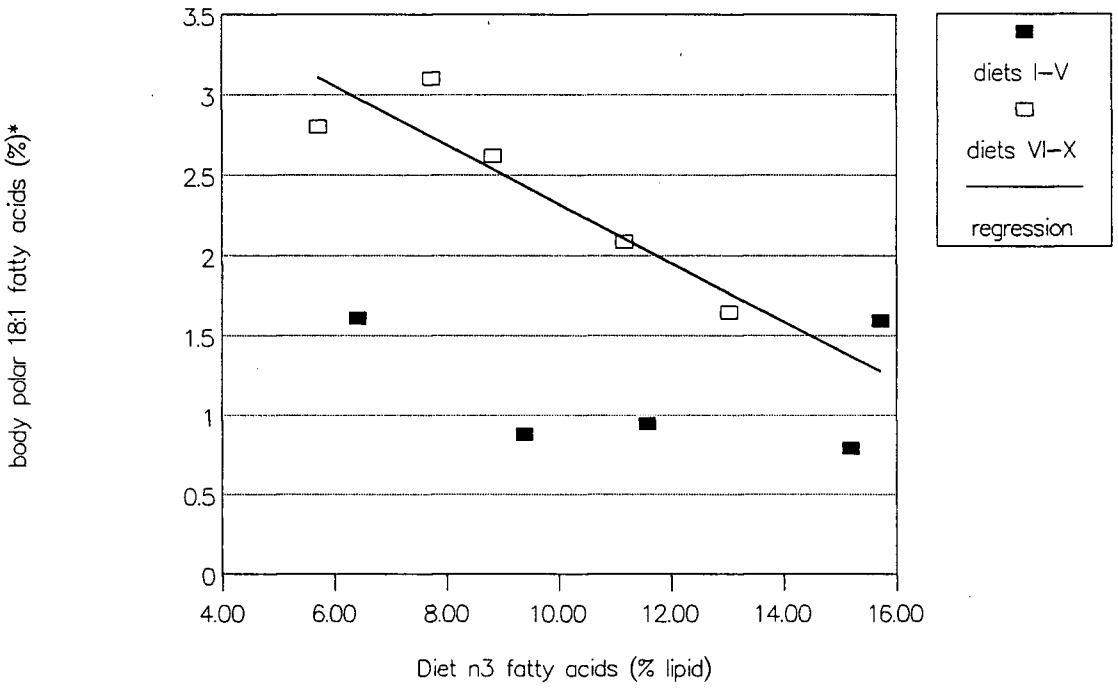

Figure 21. 18:1 fatty acid concentration in the body polar lipid as a function of dietary n3 fatty acid concentration (% lipid) in period 1. The regression involves the high lipid diets (VI-X) only.
*body fatty acids expressed as % PFA (see page 57)
$y = -0.18x + 4.16$ $r^2 = 0.82$

IV. Total n3 fatty acids

There were significant differences between elevations of high and low lipid groups for the regressions of body polar lipid as a function of dietary n3 (% DMB and % lipid).

There were no significant slopes produced by the regressions of body n3 content, either neutral or polar, as a function of dietary n3 (% DMB or % lipid).

V. n3 highly unsaturated fatty acids (n3 HUFA's)

There were no significant slopes produced by any of the regressions for body n3 HUFA in either the body neutral or polar lipid fractions. This was true for both independent variables , diet n3 and diet n3 HUFA regardless of the method in which they were quantified (% DMB or % lipid).

VI. Total n6 fatty acids

There were no significant slopes produced for regressions of the n6 concnetration in the body neutral lipid as a function of either dietary n3 or dietary n6. This was true regardless of the method in which the independent variables were quantified.

Significant differences between elevations of high and low lipid groups for the regressions of n6 concentration in the body polar lipid as a function of dietary n3 (% DMB and % lipid) or dietary n6 (% lipid).

The regression of n6 fatty acid content in the body polar lipid as a function of dietary n6 (% DMB) produced a significant slope. This regression is shown in figure 22. The r^2 value for this regression is 0.71.

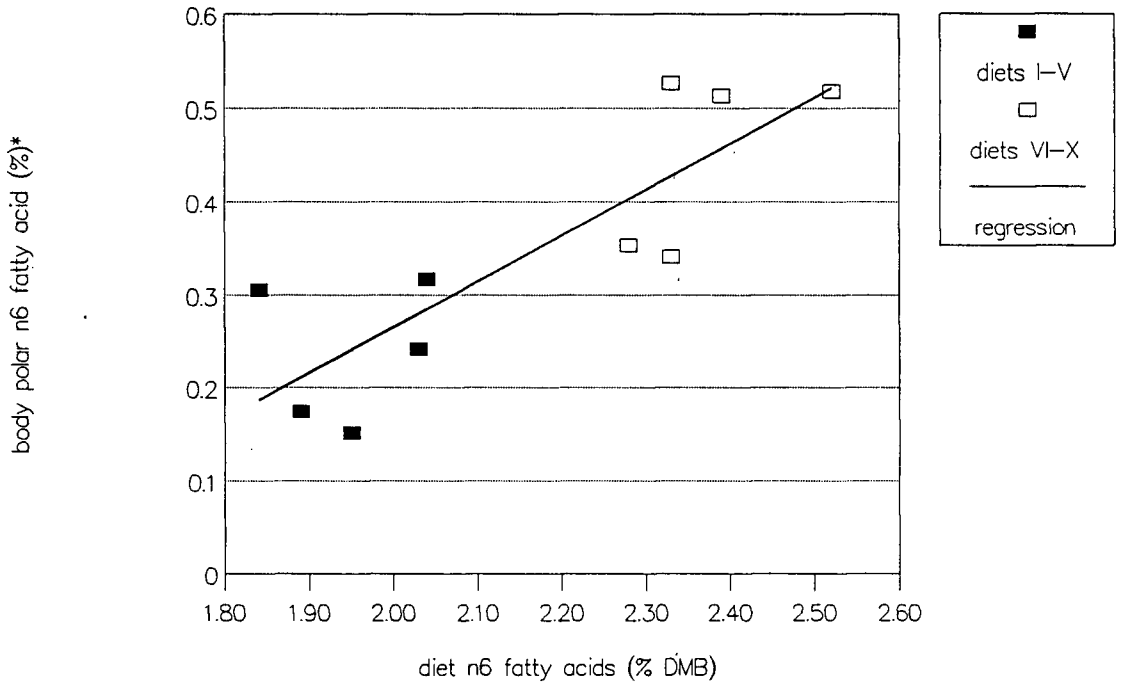

Figure 22. n6 fatty acid concentration in the body polar lipid as a function of dietary n6 fatty acid concentration (% DMB) in period 1.
The regression involves all diets (I-X).
*body fatty acids expressed as % PFA (see page 57)
$y = 0.49x - 0.72$ $r^2 = 0.71$

Summary of dietary effects on body composition during period 1

Saturated fatty acid content in the body neutral lipid decreased with increased dietary saturated (% lipid) or n3 fatty acids (% lipid).

Increases in dietary monounsaturates, either as percent of the diet or of dietary lipid increased the monounsaturate content in the body neutral lipid. An increase in dietary n3 (% lipid) decreased the monounsaturate concentration in the body neutral lipid.

Increases in the dietary monounsaturate content (% DMB) resulted in increased monounsaturates in the polar lipid fraction. Increases in the dietary n3 (% DMB or % lipid) resulted in decreases in the monounsaturated fatty acid concnetration in the body polar lipid of the high dietary lipid groups.

Increases in the c18 monounsaturates, both as a percent of the diet or of the dietary lipid, increased the 18:1 fatty acid content in the body neutral lipid. Increases in dietary n3 (% lipid) caused a decrease in the 18:1 content in the body neutral lipid. The 18:1 concentration in the body neutral lipid decreased in the low lipid group when dietary n3 (% DMB) increased. Increased dietary n3 (% lipid) decreased the concentration of 18:1 fatty acids in the body polar lipid of the high

dietary lipid treatment group.

Dietary n3 content had no significant effect on n3 content in either the neutral or polar lipid fractions in the body.

Body n3 HUFA concentration was not significantly influenced by changes in dietary n3 HUFA or total n3 concentrations.

n6 fatty acid concentration in the body polar lipid increased as dietary n6 concentration increased (% DMB).

Table 11

Statistically significant regressions of body fatty acid composition as a function of dietary fatty acids from period 1.

Variables					
Dependent			Independent		
class	lipid	group**	class	regression	r^2
sats*	N*	low	sats(% DMB)	y = -1.34x+7.74	0.83
sats	N	high	sats(% DMB)***	y = -0.53x+6.57	0.09
sats	N	pool	sats(%lipid)	y = -0.30x+8.88	0.54
sats	N	pool	n3 (%lipid)	y = 0.18x+5.98	0.47
sats	N	pool	18:1(% DMB)	y = 0.23x+2.05	0.50
sats	N	pool	18:1(%lipid)	y = 0.09x+0.59	0.49
sats	N	pool	mono(% DMB)	y = 0.22x+1.61	0.51
sats	N	pool	mono(%lipid)	y = 0.09x-0.77	0.50
mono	N	pool	mono(% DMB)	y = 0.95x+2.79	0.66
mono	N	pool	mono(%lipid)	y = 0.37x-6.10	0.54
mono	P	pool	mono(% DMB)	y = 0.35x-0.63	0.63
mono	N	pool	n3 (%lipid)	y = -0.71x+21.30	0.49
mono	P	high	n3 (% DMB)	y = -1.18x+7.06	0.79
mono	P	low	n3 (% DMB)***	y = -0.08x+2.63	0.00
mono	P	high	n3 (%lipid)	y = -0.31x+5.57	0.81
mono	P	low	n3 (%lipid)***	y = -0.02x+2.73	0.01
18:1	N	pool	18:1(% DMB)	y = 0.77x+0.05	0.64
18:1	N	pool	18:1(%lipid)	y = 0.27x-4.29	0.57
18:1	N	low	n3 (% DMB)	y = -1.75x+8.86	0.85
18:1	N	high	n3 (% DMB)***	y = -2.01x+13.24	0.32
18:1	N	pool	n3 (%lipid)	y = -0.54x+12.53	0.50
18:1	P	high	n3 (% DMB)	y = -0.71x+4.09	0.80
18:1	P	low	n3 (% DMB)***	y = -0.09x+1.35	0.03
18:1	P	high	n3 (%lipid)	y = -0.18x+4.16	0.82
18:1	P	low	n3 (%lipid)***	y = -0.02x+1.40	0.04
n6	P	pool	n6 (% DMB)	y = 0.49x-0.72	0.71

*sat = total saturated fatty acids
mono = total monounsaturated fatty acids
18:1 = C18 monounsaturated fatty acids
N = body neutral lipid fraction
P = body polar lipid fraction

**group = treatment group involved in the regression
low = a regression using the low lipid diets (I - V) only
high = a regression using the high lipid diets(VI- X) only
pool = a regression using all diets (I - X)

***This regression did not have a slope that was significantly different from zero (P>0.05).

B. The fatty acid composition of fish sampled in Period 2.

Table 12

Body dry matter and lipid composition at the end of period 2.

Concentrations of polar and neutral body lipid are expressed as percentages of the total lipid or of the non-lipid dry structural body weight.

Diet		% DM*	% lipid DMB	lipid % polar	lipid % neutral	polar/ nonlipid dry b.wt (%)	neutral/ nonlipid dry b.wt+ polar (%)
I	a	27.41	38.60	17.62	82.38	11.08	46.62
	b	28.94	34.10	17.49	82.51	9.05	39.16
II	a	23.64	34.43	22.93	77.07	12.04	36.12
	b	33.14	30.89	22.28	77.72	9.19	29.37
III	a	29.23	34.11	20.81	79.19	10.37	35.75
	b	19.89	37.12	30.30	69.70	17.87	34.88
IV	a	24.55	30.83	23.94	76.06	10.67	30.64
	b	30.03	39.03	14.49	85.51	9.27	50.09
V	a	27.43	34.05	19.49	80.51	10.06	37.77
	b	26.99	29.94	18.75	81.25	8.01	32.14
VI	a	33.76	31.34	13.76	86.24	6.28	37.04
	b	30.05	46.46	10.67	89.33	9.26	70.94
VII	a	30.32	35.36	18.98	81.02	10.38	40.15
	b	27.44	38.67	14.23	85.77	8.97	49.62
VIII	a	36.36	35.51	22.22	77.78	12.23	38.15
	b	29.88	48.06	13.16	86.84	12.18	71.63
IX	a	29.03	40.27	9.05	90.95	6.10	57.79
	b	31.50	39.78	13.24	86.76	8.75	52.70
X	a	21.15	24.73	27.60	72.40	9.07	21.81
	b	26.22	40.24	20.00	80.00	13.47	47.47

* DM = dry matter

Table 13

Fatty acid composition of polar lipid extracted from fish at the end of period 2.

Concentrations of fatty acids are expressed as percentages of the polar lipid.

Classes of fatty acids

Diet	Rep	sat*	monounsat*	18:1	n3	n3 HUFA*	n6	n6 HUFA*
I	a	20.82	38.82	28.92	21.76	20.48	12.56	4.20
	b	22.06	43.06	32.98	20.70	20.70	11.49	2.91
II	a	23.84	36.65	28.76	29.79	29.79	9.72	1.97
	b	23.56	38.01	29.37	26.14	25.48	10.99	2.44
III	a	20.27	37.12	27.55	28.83	27.68	10.48	2.95
	b	17.79	33.03	24.56	26.63	25.31	11.38	4.01
IV	a	25.52	32.40	24.11	32.52	32.52	8.27	1.50
	b	27.37	38.50	26.33	26.41	26.41	7.71	ND**
V	a	24.77	27.85	20.74	34.82	33.69	9.14	2.29
	b	20.84	29.55	19.15	36.81	35.73	8.82	3.33
VI	a	19.03	46.82	35.57	20.97	20.62	10.45	2.17
	b	20.99	44.29	30.77	24.30	24.30	10.42	3.55
VII	a	-	-	-	-	-	-	-
	b	19.70	43.38	35.18	20.74	20.74	12.05	4.57
VIII	a	21.94	43.04	34.92	25.51	24.56	8.01	1.90
	b	23.42	44.12	34.46	19.95	19.08	8.67	0.90
IX	a	25.91	34.83	26.63	31.85	31.85	7.41	ND
	b	28.97	32.17	23.35	31.25	31.25	6.25	ND
X	a	18.17	36.30	25.27	25.73	24.69	8.44	2.20
	b	23.95	43.69	35.14	25.76	25.76	6.60	ND

* sat = total of all saturated fatty acids
monounsat = total of all monounsaturated fatty acids
n3 HUFA = total of all n3 fatty acids > C18
n6 HUFA = total of all n6 fatty acids > C18
** ND = not detected

Table 14

Fatty acid composition of polar lipid extracted from fish at the end of period 2.

Concentrations of fatty acids are expressed as percentages of the non-lipid dry body weight.

		Classes of fatty acids						
Diet	Rep	sat*	monounsat*	18:1	n3	n3 HUFA*	n6	n6 HUFA*
		%	%	%	%	%	%	%
I	a	2.31	4.30	3.20	2.41	2.27	1.39	0.47
	b	2.00	3.90	2.99	1.87	1.87	1.04	0.26
II	a	2.87	4.41	3.46	3.59	3.59	1.17	0.24
	b	2.17	3.49	2.70	2.40	2.34	1.01	0.22
III	a	2.10	3.85	2.86	2.99	2.87	1.09	0.31
	b	3.18	5.90	4.39	4.76	4.52	2.03	0.72
IV	a	2.72	3.46	2.57	3.47	3.47	0.88	0.16
	b	2.54	3.57	2.44	2.45	2.45	0.72	ND**
V	a	2.49	2.80	2.09	3.50	3.39	0.92	0.23
	b	1.67	2.37	1.53	2.95	2.86	0.71	0.27
VI	a	1.20	2.94	2.23	1.32	1.30	0.66	0.14
	b	1.94	4.10	2.85	2.25	2.25	0.96	0.33
VII	a	-	-	-	-	-	-	-
	b	2.05	4.50	3.65	2.15	2.15	1.25	0.47
VIII	a	1.97	3.86	3.13	2.29	2.20	0.72	0.17
	b	2.86	5.40	4.22	2.44	2.33	1.06	0.11
IX	a	3.15	4.24	3.24	3.88	3.88	0.90	ND
	b	1.77	1.96	1.42	1.91	1.91	0.38	ND
X	a	1.59	3.17	2.21	2.25	2.16	0.74	0.19
	b	2.17	3.96	3.19	2.34	2.34	0.60	ND

* sat = total of all saturated fatty acids
monounsat = total of all monounsaturated fatty acids
n3 HUFA = total of all n3 fatty acids > C18
n6 HUFA = total of all n6 fatty acids > C18
** ND = not detected

Table 15

Fatty acid composition of neutral lipid extracted from fish at the end of period 2.

Concentrations of fatty acids are expressed as percentages of the neutral lipid.

Diet	Rep	Classes of fatty acids						
		sat*	monounsat*	18:1	n3	n3 HUFA*	n6	n6 HUFA*
		%	%	%	%	%	%	%
I	a	18.84	58.83	47.61	4.89	4.05	11.98	0.63
	b	17.66	55.39	44.91	6.63	5.05	12.53	1.68
II	a	17.46	55.08	41.69	8.88	7.26	12.55	1.43
	b	18.86	56.04	44.17	6.92	5.78	12.30	0.59
III	a	19.65	55.20	43.09	10.19	8.48	11.86	ND**
	b	17.65	52.70	40.83	10.55	8.98	12.52	0.84
IV	a	18.62	51.40	36.77	12.79	10.80	11.73	0.30
	b	20.94	48.89	35.50	11.31	9.71	9.33	ND
V	a	23.11	47.63	35.33	13.54	11.40	11.67	0.35
	b	20.84	49.25	35.61	14.54	12.50	11.67	0.60
VI	a	14.70	61.46	49.62	4.96	4.30	10.52	0.96
	b	18.39	66.50	56.47	3.44	3.44	10.34	ND
VII	a	15.44	59.61	48.67	6.08	5.01	11.45	0.82
	b	15.24	61.27	49.16	6.78	5.62	10.09	0.85
VIII	a	18.74	59.49	48.06	8.11	6.69	9.53	ND
	b	15.66	52.07	40.53	9.27	7.80	11.83	3.21
IX	a	17.12	58.15	45.38	9.14	7.72	9.71	0.32
	b	16.27	56.42	45.38	9.61	8.17	10.94	0.59
X	a	20.69	46.52	33.57	15.59	13.40	11.88	0.35
	b	18.57	58.51	46.92	8.85	7.91	10.02	ND

* sat = total of all saturated fatty acids
monounsat = total of all monounsaturated fatty acids
n3 HUFA = total of all n3 fatty acids > C18
n6 HUFA = total of all n6 fatty acids > C18
** ND = not detected

Table 16.

Fatty acid composition of neutral lipid extracted from fish at the end of period 2.

Concentrations of fatty acids are expressed as percentages of the non-lipid dry structural body weight.

		Classes of fatty acids						
Diet	Rep	sat*	monounsat*	18:1	n3	3 HUFA*	n6	n6 HUFA*
		%	%	%	%	%	%	%
I	a	9.76	30.47	24.66	2.53	2.10	6.20	0.33
	b	7.54	23.65	19.18	2.83	2.16	5.35	0.72
II	a	7.07	22.29	16.87	3.59	2.94	5.08	0.58
	b	6.05	17.97	14.16	2.22	1.85	3.94	0.19
III	a	7.75	21.78	17.00	4.02	3.35	4.68	ND**
	b	7.26	21.67	16.79	4.34	3.69	5.15	0.35
IV	a	6.31	17.43	12.47	4.34	3.68	3.98	0.10
	b	11.46	26.76	19.43	6.19	5.31	5.11	ND
V	a	9.61	19.80	14.69	5.63	4.73	4.85	0.15
	b	7.24	17.10	12.36	5.05	4.35	4.05	0.21
VI	a	5.79	24.19	19.53	1.95	1.69	4.14	0.38
	b	14.25	51.54	43.77	2.67	2.67	8.01	ND
VII	a	6.84	26.41	21.57	2.69	2.22	5.07	0.36
	b	8.24	33.13	26.58	3.67	3.04	5.46	0.46
VIII	a	8.02	25.47	20.58	3.47	2.86	4.08	ND
	b	12.58	41.84	32.57	7.45	6.27	9.51	2.58
IX	a	10.50	35.65	27.82	5.60	4.73	5.95	0.20
	b	9.32	32.33	26.01	5.51	4.68	6.27	0.34
X	a	4.92	11.06	7.98	3.71	3.19	2.83	0.08
	b	10.00	31.51	25.27	4.77	4.26	5.40	ND

* sat = total of all saturated fatty acids
monounsat = total of all monounsaturated fatty acids
n3 HUFA = total of all n3 fatty acids > C18
n6 HUFA = total of all n6 fatty acids > C18
** ND = not detected

I. Saturated fatty acids

The content of saturated fatty acids in either the polar or the neutral fractions of body lipid did not produce a statistically significant slope when related to dietary saturated or dietary n3 fatty acids. This lack of significance was not altered by method of quantifying the dietary fatty acid calculated as % of dietary lipid or as % of the diet on a dry matter basis.

II. Monounsaturated fatty acids

A single regression for the relation of monounsaturated fatty acid concentration in the body neutral lipid to dietary n3 (% DMB) could not be produced because of a significant difference in elevations of the low and high dietary lipid treatment groups. The individual regressions for each of these groups did not produce significant slopes. There were significant slopes produced when the monounsaturated fatty acid content in the body neutral lipid was associated with dietary monounsaturates (% DMB and % dietary lipid) and the dietary n3 (% of dietary lipid). These regressions are shown in figures 23, 24 and 25, respectively.

A significant slope was not produced by any of the independent variables when the monounsaturated fatty acid content in the body polar lipid was analyzed.

The r^2 varied only slightly for the relation of neutral body monounsaturate content as a function of either dietary n3 or monounsaturate (% dietary lipid). The r^2 values were 0.24 and 0.27 respectively.

There was only a small difference in the r^2 values for monounsaturated fatty acid concentration in the body neutral lipid as a function of the monounsaturate in the diet (% DMB) or in the lipid (%lipid). The values were 0.34 and 0.27 respectively.

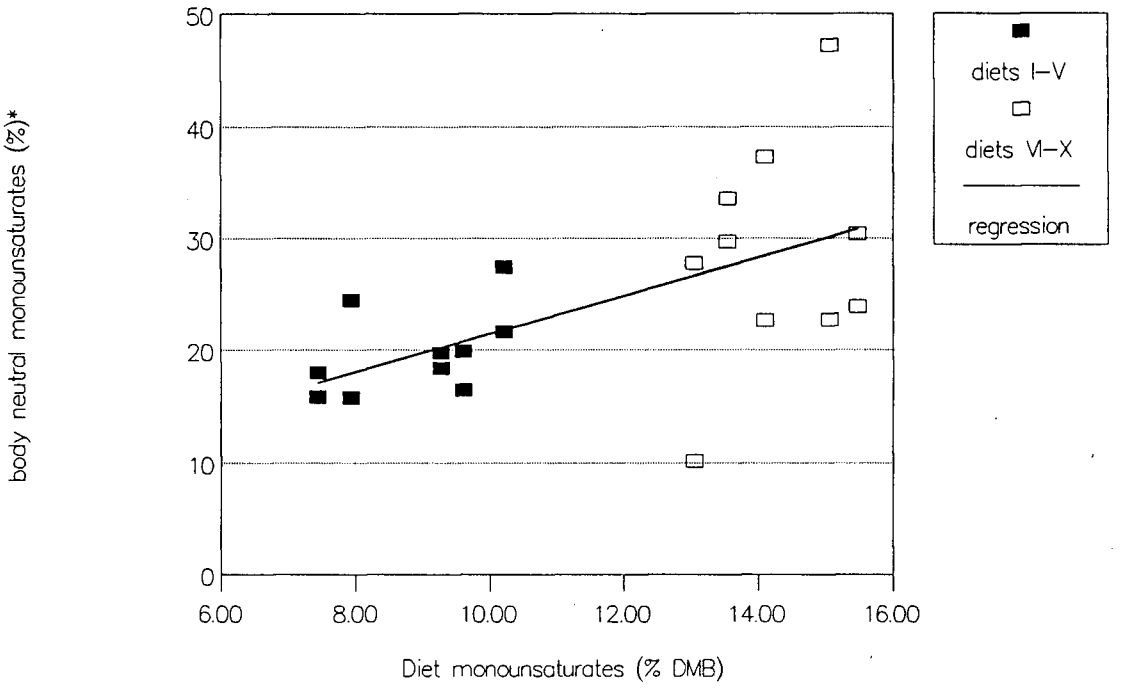

Figure 23. Monounsaturated fatty acid concentration in the body neutral lipid as a function of dietary monounsaturated fatty acid concentration (% DMB) in period 2.
The regression involves all diets (I-X).
*body fatty acids expressed as % NFA (see page 56)
$y = 1.71x + 4.37$ $r^2 = 0.34$

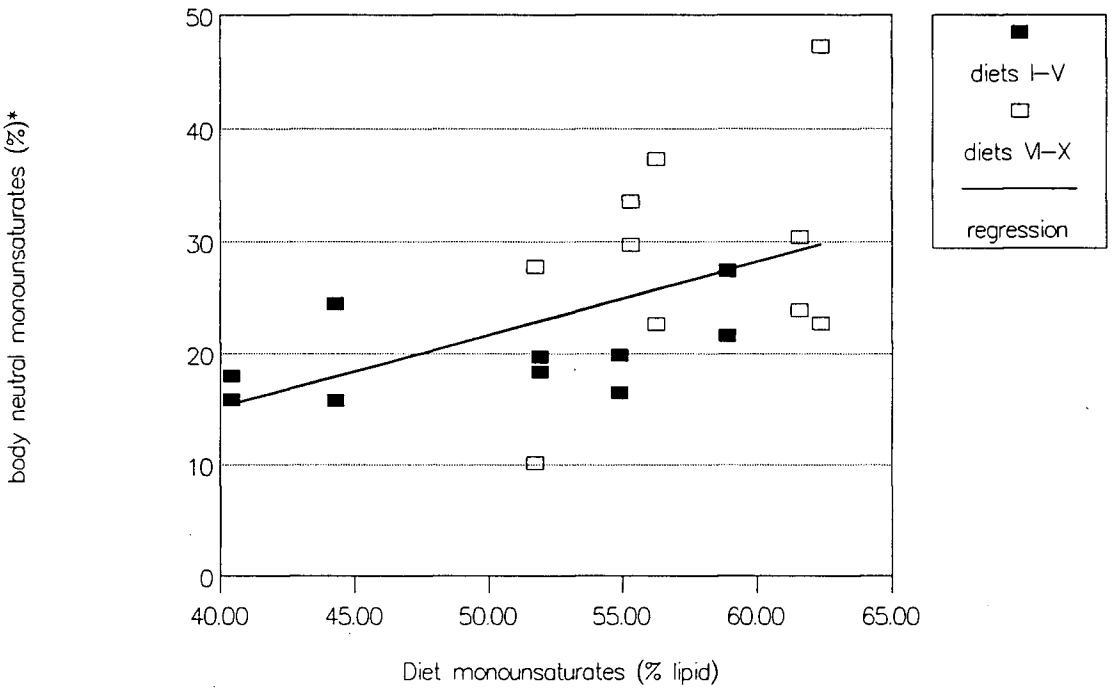

Figure 24. Monounsaturated fatty acid concentration in the body neutral lipid as a function of dietary monounsaturated fatty acid concentration (% lipid) in period 2.
The regression involves all diets (I-X).
*body fatty acids expressed as % NFA (see page 56)
$y = 0.66x - 11.20$ $r^2 = 0.27$

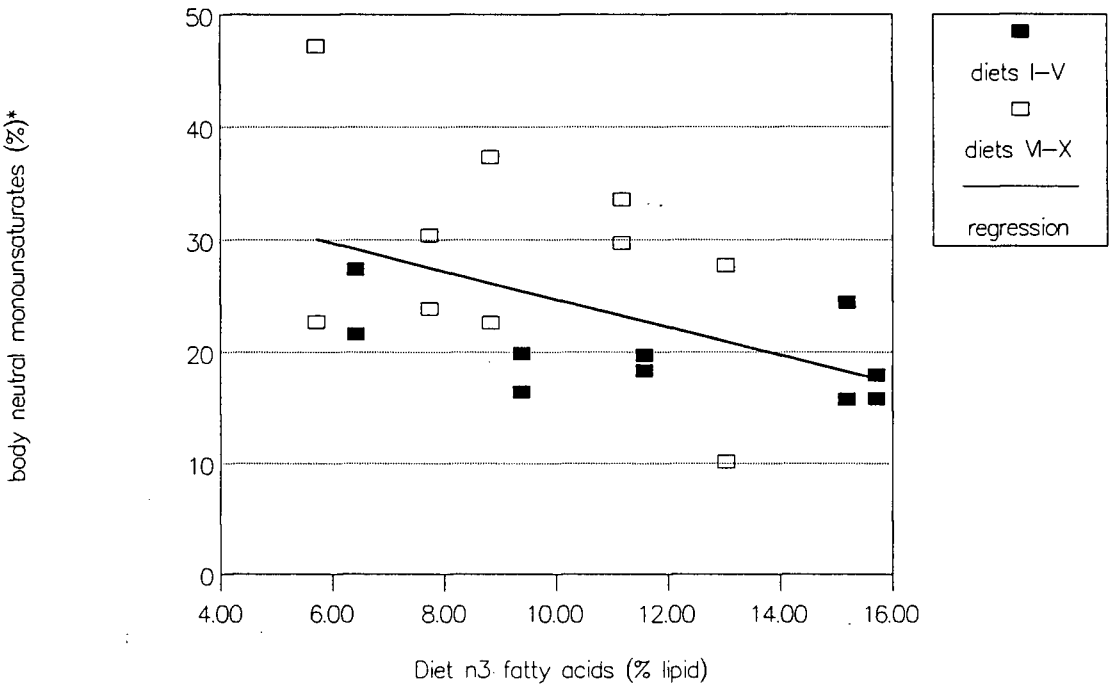

Figure 25. Monounsaturated fatty acid concentration in the body neutral lipid as a function of dietary n3 fatty acid concentration (% lipid) in period 2. The regression involves all diets (I-X).
*body fatty acids expressed as % NFA (see page 56)
$y = -1.24x + 37.20$ $r^2 = 0.24$

III. C18 Monounsaturated fatty acids

Once again there were significant differences in the elevations of the low and high lipid groups when the body neutral 18:1 content was related to the diet n3 (% DMB). The regression involving the low dietary lipid group produced a significant slope with an r^2 value of 0.41. The regression is shown in figure 29. Significant slope were also produced by the regressions of 18:1 fatty acids in the body neutral lipid and dietary 18:1 (% DMB and % lipid). These regressions are shown in figures 26 and 27, respectively.

The elevations between high and low groups were also significantly different when the 18:1 fatty acids in the body polar lipid was related to dietary 18:1 (% of dry diet). The regression involving the low dietary lipid group (figure 28) produced a significant slope with an r^2 value of 0.58.

The r^2 value was slightly higher when the neutral body 18:1 was associated with diet 18:1 (% lipid) rather than diet n3 (% lipid). The r^2 values were 0.37 and 0.29, respectively. There was very little difference between the r^2 values of 18:1 concentration in the body neutral lipid as a function of 18:1 in the diet expressed as % DMB

or as % lipid. The r^2 values were 0.38 and 0.37 respectively.

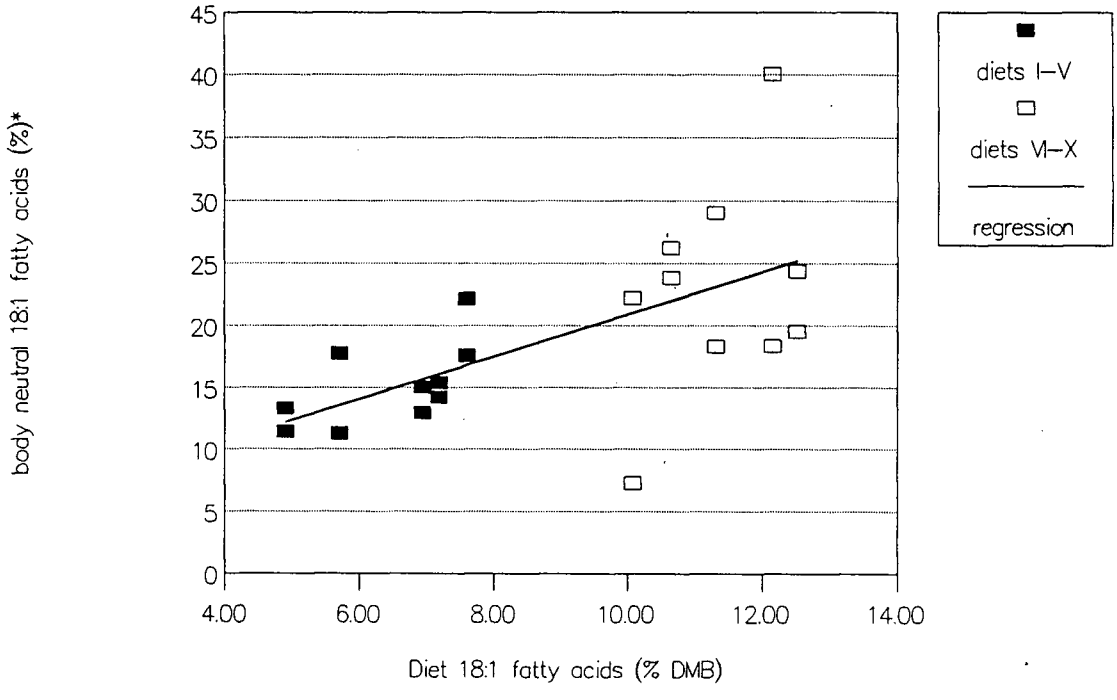

Figure 26. 18:1 fatty acid concentration in the body neutral lipid as a function of dietary 18:1 fatty acid concentration (% DMB) in period 2. The regression involves all diets (I-X).
*body fatty acids expressed as % NFA (see page 56)
$y = 1.71x + 3.80$ $r^2 = 0.38$

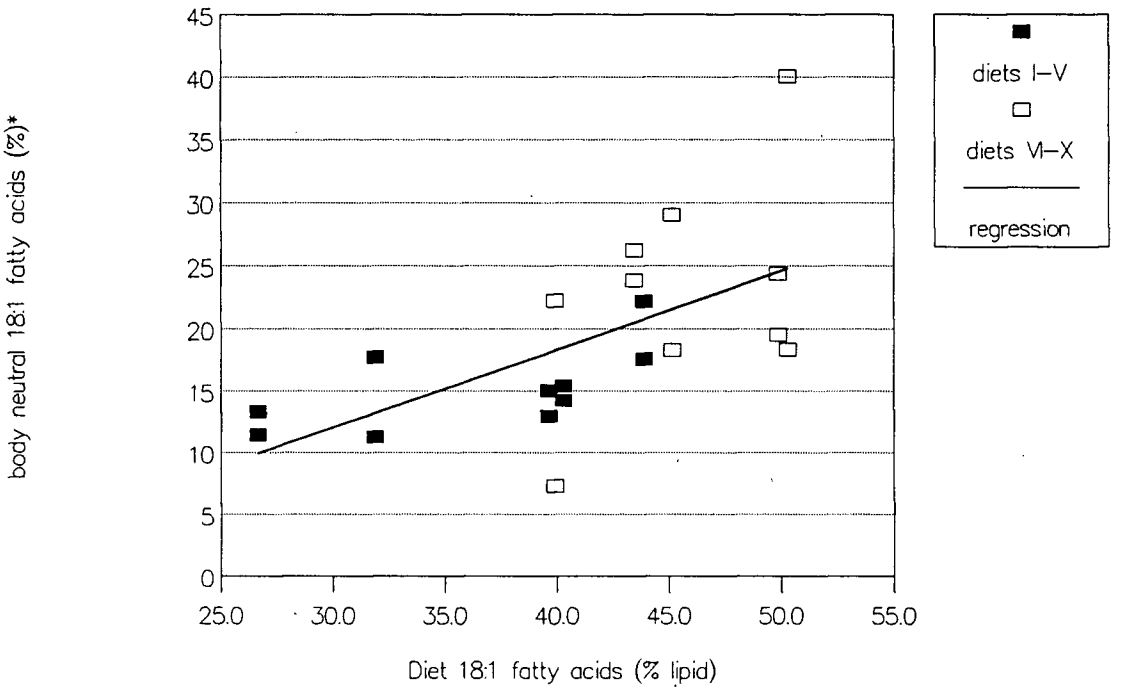

Figure 27. 18:1 fatty acid concentration in the body neutral lipid as a function of dietary 18:1 fatty acid concentration (% lipid) in period 2. The regression involves all diets (I-X).
*body fatty acids expressed as % NFA (see page 56)
$y = 0.63x - 6.81$ $r^2 = 0.37$

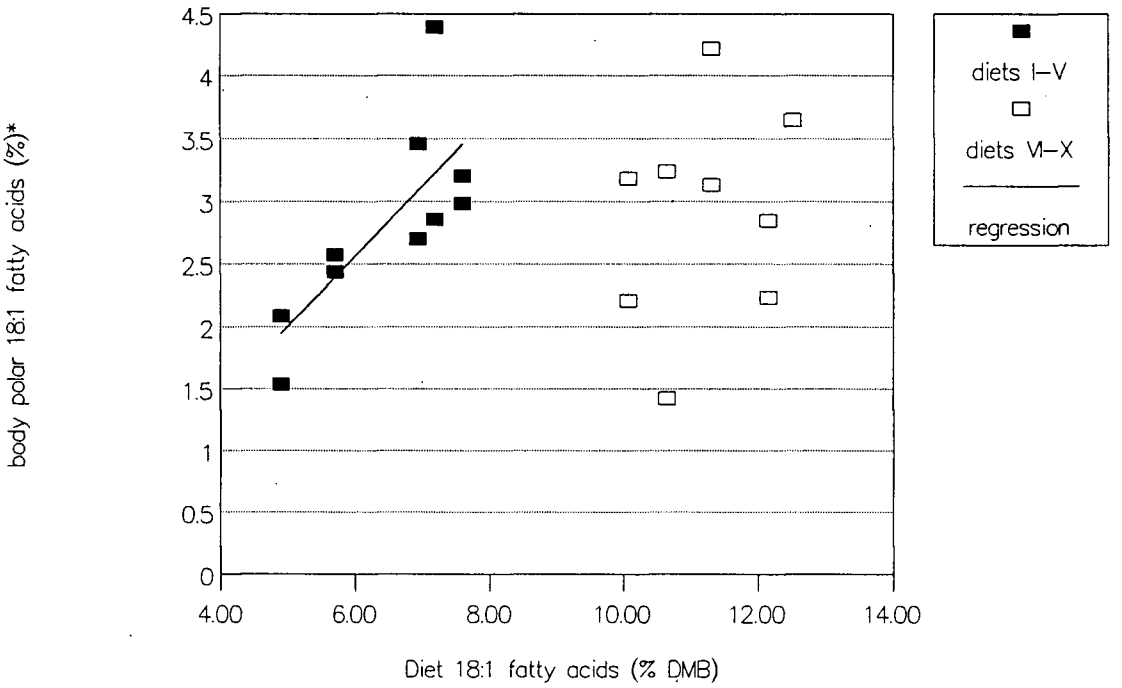

Figure 28. 18:1 fatty acid concentration in the body polar lipid as a function of dietary 18:1 fatty acid concentration (% DMB) in period 2. The regression involves the low lipid diets (I-V) only.
*body fatty acids expressed as % PFA (see page 57)
$y = 0.56x - 0.79$ $r^2 = 0.58$

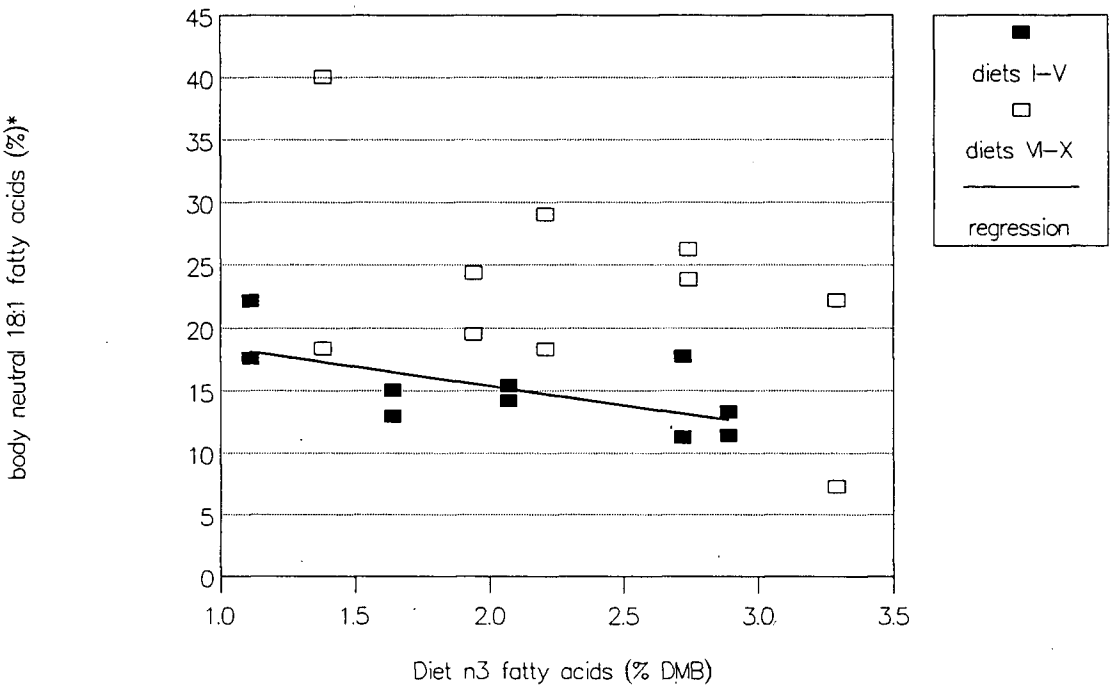

Figure 29. 18:1 fatty acid concentration in the body neutral lipid as a function of dietary n3 fatty acid concentration (% DMB) in period 2. The regression involves the low lipid diets (I-V) only.
*body fatty acids expressed as % NFA (see page 56)
$y = -3.06x + 21.51$ $r^2 = 0.41$

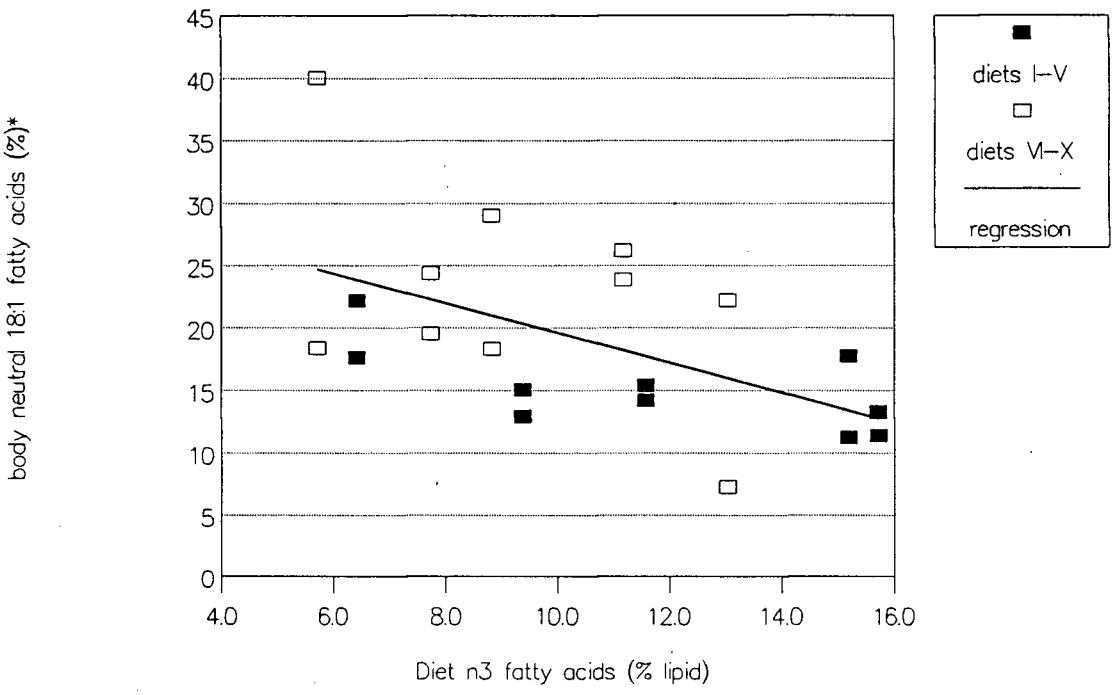

Figure 30. 18:1 fatty acid concentration in the body neutral lipid as a function of dietary n3 fatty acid concentration (% lipid) in period 2. The regression involves all diets (I-X).
*body fatty acids expressed as % NFA (see page 56)
$y = -1.19x + 31.53$ $r^2 = 0.29$

IV. Total n3 fatty acids

There was a significant difference in elevation between the low and high lipid groups when n3 fatty acid concentration in the body polar lipid was related to dietary n3 fatty acids (%DMB).

There was only a slight difference in the r^2 for body neutral n3 as a function of diet n3 as a percent of the diet (DMB) or of the dietary lipid. The r^2 values were 0.44 and 0.39, respectively. Figures 31 and 32 show these regressions.

A significant slope was produced when the n3 fatty acid content in the body polar lipid was related to diet n3 (% lipid). This regression produced an r^2 value of 0.22. It is shown in figure 33.

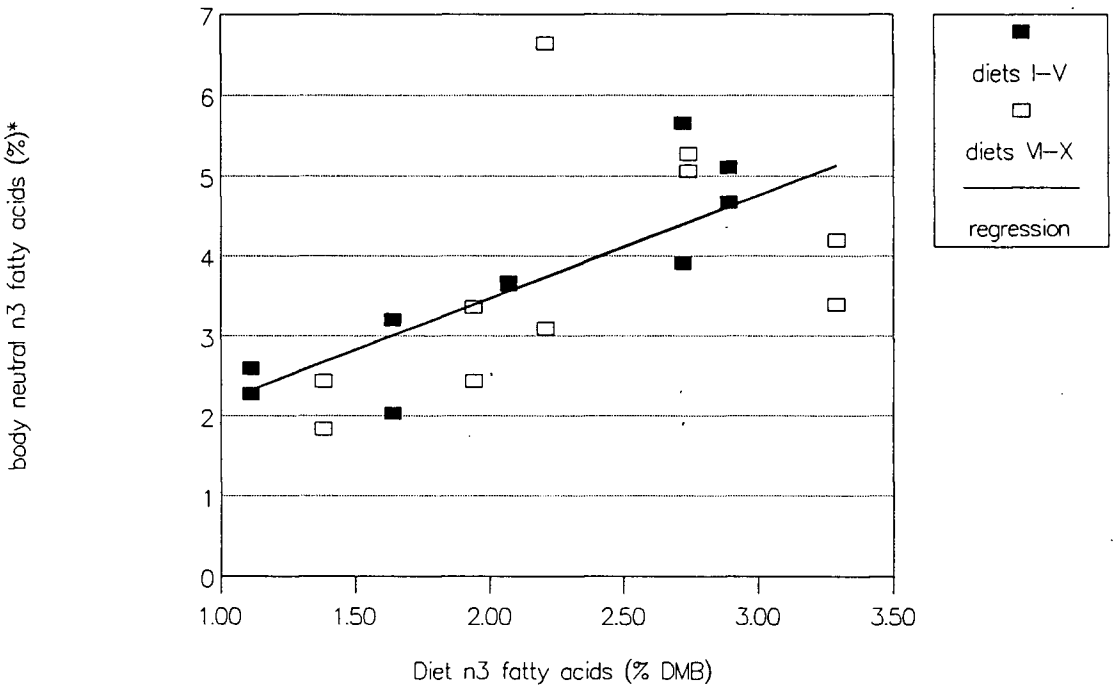

Figure 31. n3 fatty acid concentration in the body neutral lipid as a function of dietary n3 fatty acid concentration (% DMB) in period 2. The regression involves all diets (I-X).
*body fatty acids expressed as % NFA (see page 56)
$y = 1.29x + 0.89$ $r^2 = 0.44$

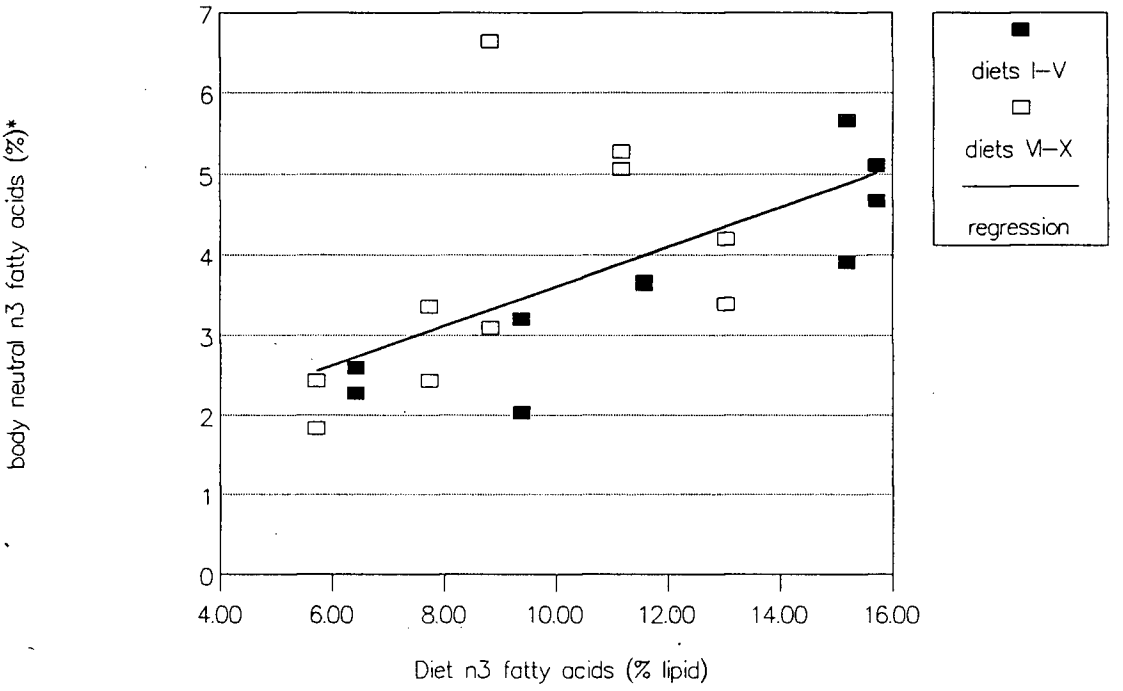

Figure 32. n3 fatty acid concentration in the body neutral lipid as a function of dietary n3 fatty acid concentration (% lipid) in period 2. The regression involves all diets (I-X).
*body fatty acids expressed as % NFA (see page 56)
$y = 0.25x + 1.16$ $r^2 = 0.39$

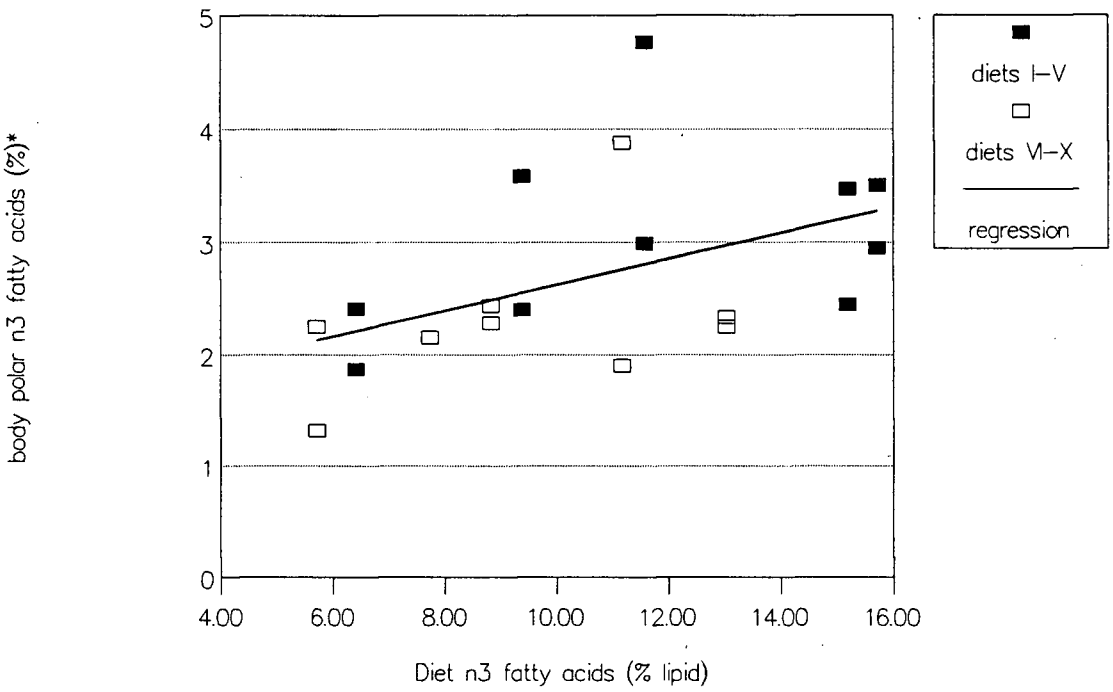

Figure 33. n3 fatty acid concentration in the body polar lipid as a function of dietary n3 fatty acid concentration (% lipid) in period 2.
The regression involves all diets (I-X).
*body fatty acids expressed as % PFA (see page 57)
$y = 0.11x + 1.48$ $r^2 = 0.22$

V. n3 highly unsaturated fatty acids (n3 HUFA's)

There were significant differences in elevation between the low and high lipid groups when n3 HUFA concentration in the body polar lipid was related to diet n3 (%DMB) or n3 HUFA (% DMB).

There was only a slight difference in the r^2 for body neutral n3 HUFA as a function of diet n3 HUFA or diet total n3 when referred to as either percent of the diet (DMB) or of the dietary lipid. The r^2 values were 0.46 and 0.47 respectively when the independent variable was calculated as a percent of the diet (DMB). The regressions produced r^2 values of .41 and .40 respectively when the independent variable calculated as a percent of the diet lipid. The r^2 values were consistently higher when the regressions used dietary fatty acids as a percent of the diet rather than was a percent of the lipid. Figures 34 and 35 show the regressions for n3 HUFA concentration in the body neutral lipid as a function of diet n3 HUFA (DMB), n3 HUFA (% lipid). Figures 37 and 38 show the regressions for n3 HUFA content in the body neutral lipid as a function of n3 (DMB) and n3 (% lipid), respectively.

There was only a slight difference in the r^2 for body

polar n3 HUFA as a function of diet n3 HUFA or diet total n3 calculated as a percent of the dietary lipid. The r^2 values were 0.22 and 0.23 respectively. It should be noted that n3 highly unsaturated fatty acids made up 85% of the total n3 in the lipid of all of the diets regardless of their concentration of n3 fatty acids. These regressions are shown in figures 36 and 39, respectively.

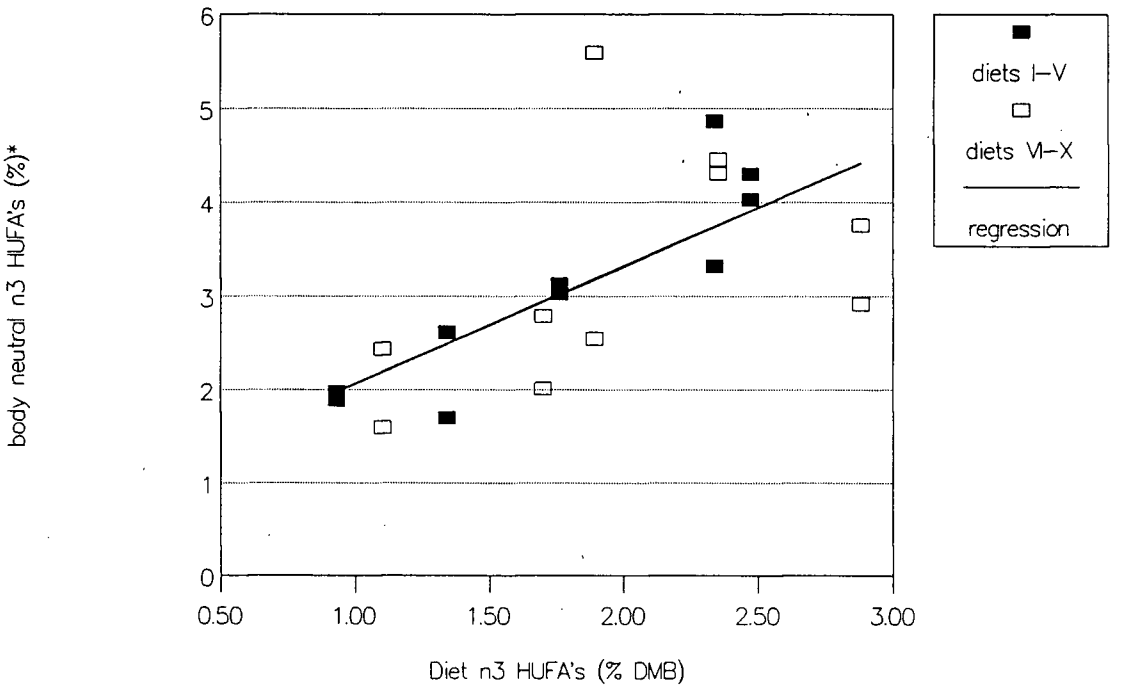

Figure 34. n3 highly unsaturated fatty acid concentration in the body neutral lipid as a function of dietary n3 highly unsaturated fatty acid concentration (% DMB) in period 2. The regression involves all diets (I-X).
*body fatty acids expressed as % NFA (see page 56)
$y = 1.25x + 0.82$ $r^2 = 0.46$

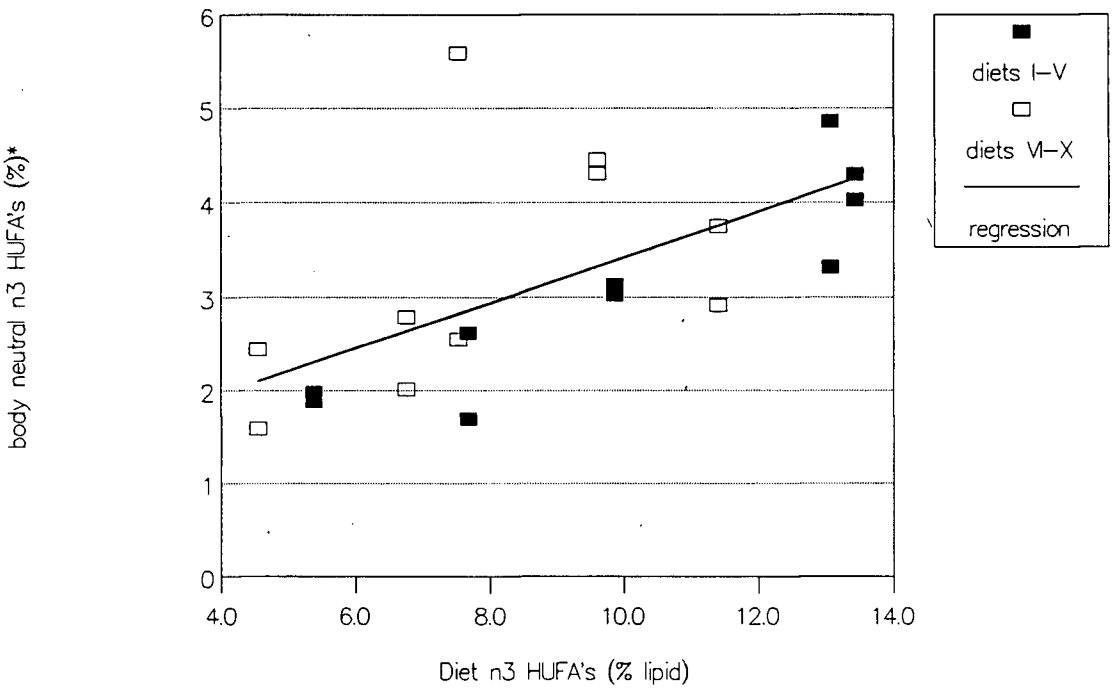

Figure 35. n3 highly unsaturated fatty acid concentration in the body neutral lipid as a function of dietary n3 highly unsaturated fatty acid concentration (% lipid) in period 2. The regression involves all diets (I-X).
*body fatty acids expressed as % NFA (see page 56)
$y = 0.24x + 1.00$ $r^2 = 0.41$

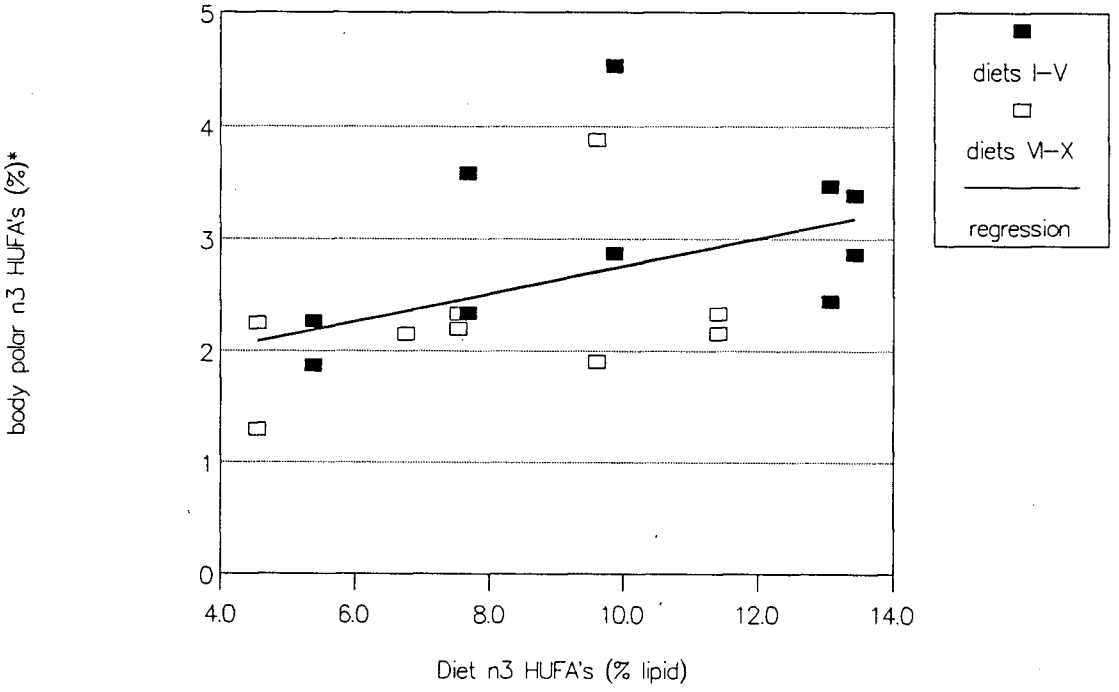

Figure 36. n3 highly unsaturated fatty acid concentration in the body polar lipid as a function of dietary n3 highly unsaturated fatty acid concentration (% lipid) in period 2. The regression involves all diets (I-X).
*body fatty acids expressed as % PFA (see page 57)
$y = 0.12x + 1.52$ $r^2 = 0.22$

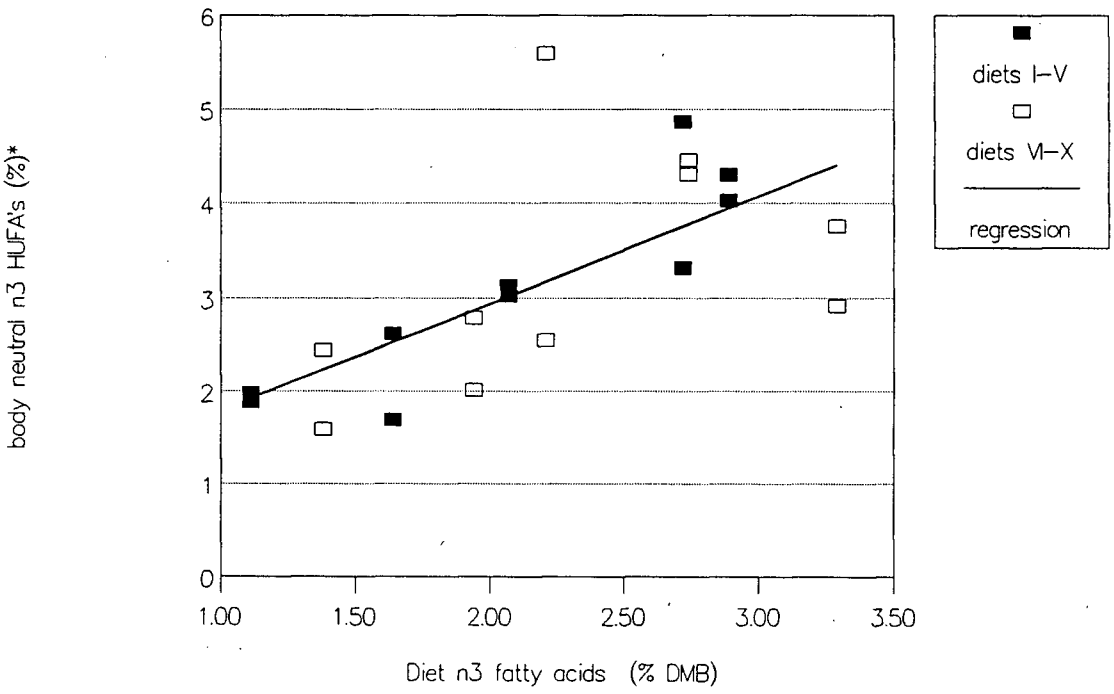

Figure 37. n3 highly unsaturated fatty acid concentration in the body neutral lipid as a function of dietary n3 fatty acid concentration (% DMB) in period 2.
The regression involves all diets (I-X).
*body fatty acids expressed as % NFA (see page 56)
$y = 1.13x + 0.67$ $r^2 = 0.47$

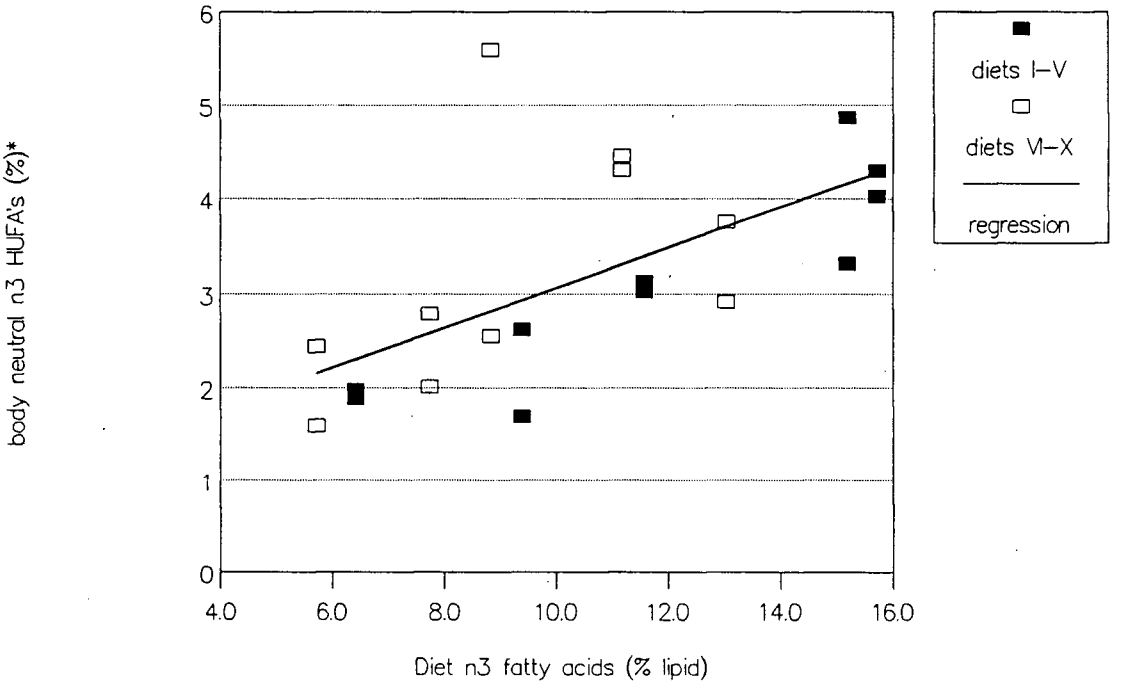

Figure 38. n3 highly unsaturated fatty acid concentration in the body neutral lipid as a function of dietary n3 fatty acid concentration (% lipid) in period 2.
The regression involves all diets (I-X).
*body fatty acids expressed as % NFA (see page 56)
$y = 0.21x + 0.94$ $r^2 = 0.40$

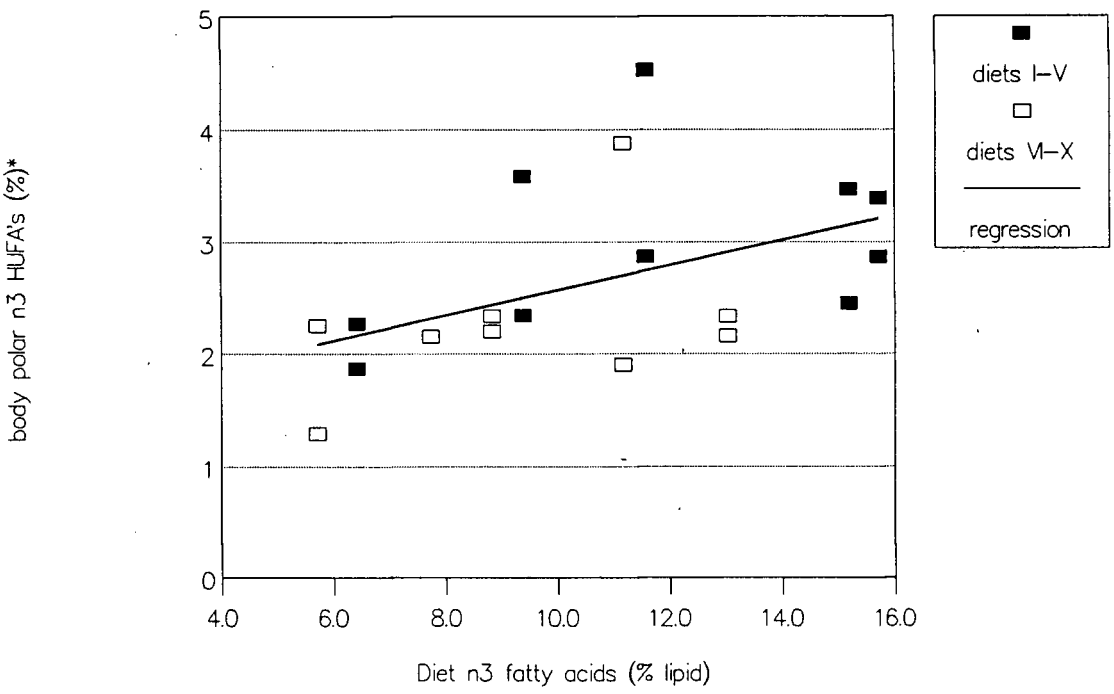

Figure 39. n3 highly unsaturated fatty acid concentration in the body polar lipid as a function of dietary n3 fatty acid concentration (% lipid) in period 2.
The regression involves all diets (I-X).
*body fatty acids expressed as % PFA (see page 57)
$y = 0.11x + 1.45$ $r^2 = 0.23$

VI. Total n6 fatty acids

There were no statistically significant slopes produced in regressions involving the neutral body n6 content.

There were significant differences in elevation between the high and low dietary lipid groups when regressions were drawn for n6 fatty acids in the body polar lipid as a function of dietary n3 (% lipid) and n6 fatty acids (% DMB). The regression of n6 fatty acid concentration in the body polar lipid as a function of the dietary n6 (% DMB) produced a significant slope in the low dietary lipid groups. The r^2 value for this regression was 0.46. The regression is shown in figure 40.

There was a significant slope produced in the regression of n6 fatty acids in the body polar lipid as a function of diet n6 (% lipid). The r^2 value for this regression was 0.39. The regression is shown in figure 41.

The regression of n6 fatty acid content in the body polar lipid as a function of diet n3 (% DMB) did not produce a significant slope.

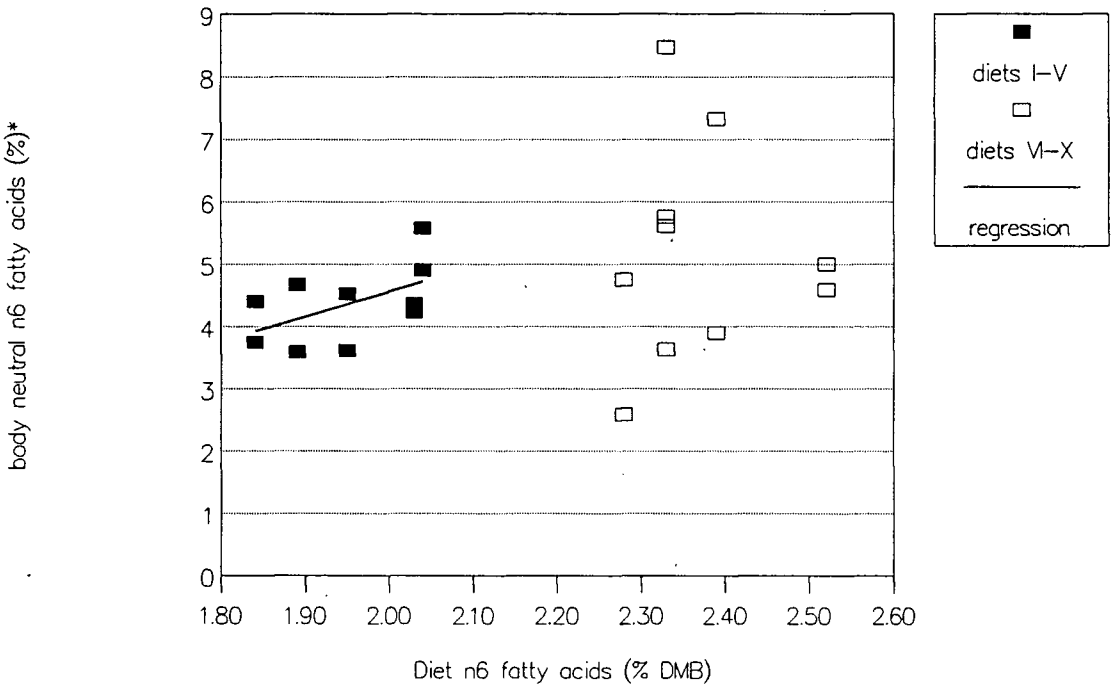

Figure 40. n6 fatty acid concentration in the body polar lipid as a function of dietary n6 fatty acid concentration (% DMB) in period 2.
The regression involves
the low lipid diets (I-V) only.
*body fatty acids expressed as % PFA (see page 57)
$y = 3.21x - 5.16$ $r^2 = 0.46$

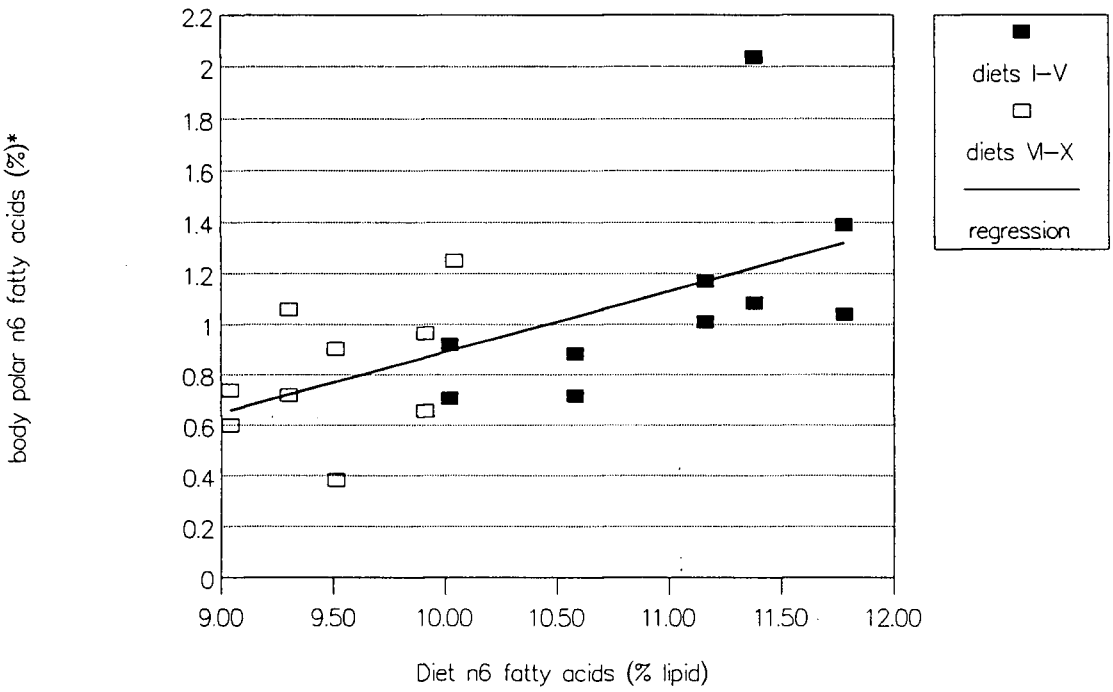

Figure 41. n6 fatty acid concentration in the body polar lipid as a function of dietary n6 fatty acid concentration (% lipid) in period 2.
The regression involves all diets (I-X).
*body fatty acids expressed as % PFA (see page 57)
$y = 0.24x - 1.52$ $r^2 = 0.39$

VII. n6 highly unsaturated fatty acids (n6 HUFA's)

No significant slopes were produced as a result of regressions of body n6 HUFA as a function of dietary n3. This was the case in both the neutral and polar body lipid fraction.

There was a significant difference in the elevation between the low and high dietary lipid groups for the regressions of n6 HUFA concentration in the body polar lipid as a function of dietary n3 (% lipid). n6 HUFA's were not found in the dietary lipid and could therefore not be used as an independent variable.

No significant slopes were produced as a result of regressions of n6 HUFA in the body neutral lipid as a function of dietary n6.

There was a significant difference in the elevation between the low and high dietary lipid groups for the regressions of n6 HUFA concentration in the body polar lipid as a function of dietary n6 (% DMB). A significant slope was produced for the regression of n6 HUFA fatty acid content in the body polar lipid as a function of dietary n6 (% lipid). The regression produced an r^2 value of 0.32. This regression is shown in figure 42.

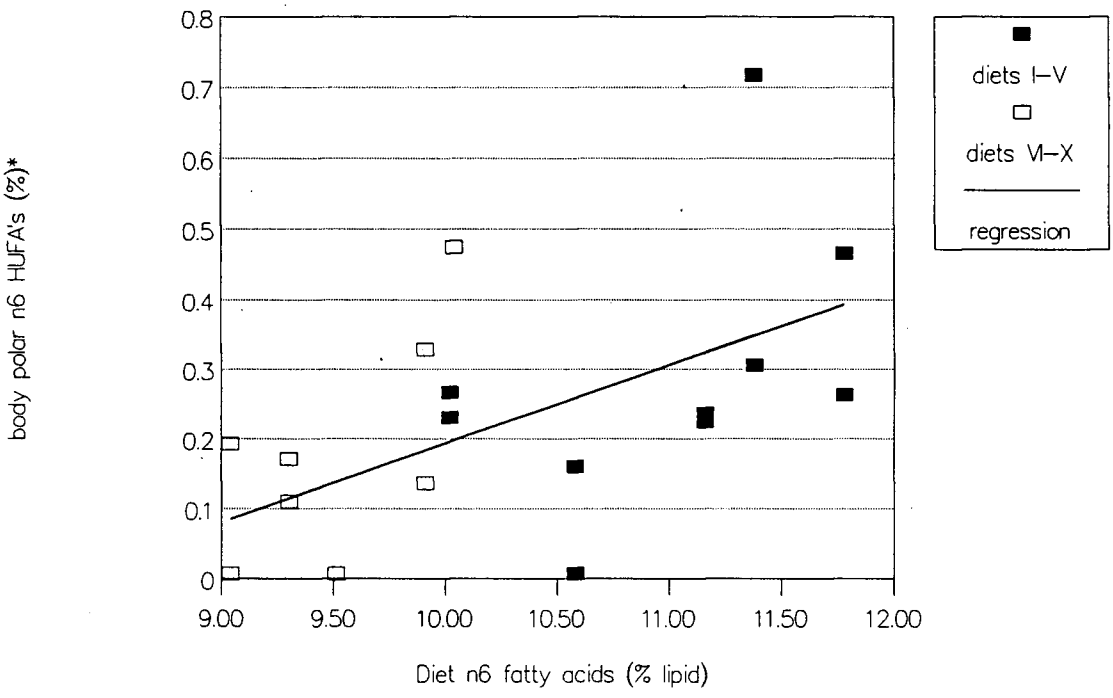

Figure 42. n6 highly unsaturated fatty acid concentration in the body polar lipid as a function of dietary n6 fatty acid concentration (% lipid) in period 2.
The regression involves all diets (I-X).
*body fatty acids expressed as % PFA (see page 57)
$y = 0.11x - 0.93$ $r^2 = 0.32$

VIII. Body lipid

The total body lipid (table 12) did not differ significantly among dietary treatments. The percentage of body polar lipid or neutral lipid over non-lipid dry body weight did not differ significantly among treatments.

IX. Body moisture

The percentage dry matter of the body was not significantly affected by dietary treatment. The data are shown in table 12.

Summary of dietary effects on body composition during period 2

From the significant regressions only a few trends in fatty acid composition can be commented on. These regressions are listed in table 17.

Increases in dietary monounsaturates, either as percent of the diet or dietary lipid increased the monounsaturate content in the body neutral lipid. An increase in dietary n3 as a percent of the lipid decreased the content of monounsaturates in the body neutral lipid.

Increases in the C18 monounsaturates, both as a percent of the diet or of the dietary lipid, increased the

18:1 fatty acid content in the body neutral lipid. Increases in dietary n3 (% lipid) caused decreases in 18:1 content in the neutral body lipid.

n3 fatty acid concentration in the body neutral lipid is increased by increasing the dietary n3 concentrations either as a percent of the diet (% DMB) or a percent of the lipid.

Increases in dietary n3 HUFA as either percent of diet (% DMB) or percent of lipid result in increased body neutral n3 HUFA content. Increases in n3 HUFA concentration in the body neutral lipid also occur as a result of increasing the total n3 in the diet (% DMB) or in the dietary lipid (% lipid). However, this may depend on the proportion of n3 HUFA in the total dietary n3. Total n3 in the body polar lipid increased as dietary total n3 (% lipid) increased although C18 n3 fatty acids only made up 2 % of the total polar n3.

n3 HUFA content in the body polar lipid increased with increases in either dietary total n3 (% lipid) or dietary n3 HUFA (% lipid).

n6 fatty acid concentration in the body polar lipid increased as dietary n6 concentration increased (%lipid).

n6 HUFA concentration in the body polar lipid also increased with increasing levels of n6 in the diet (%

lipid).

No other significant effects of dietary fatty acids were observed.

Table 17

Statistically significant regressions of body fatty acid composition as a function of dietary fatty acids from period 2.

Variables					
Dependent			Independent		
class	lipid	group**	class	regression	r^2
mono*	N*	pool	mono (% DMB)	y = 1.71x+4.37	0.34
mono	N	pool	mono (% lipid)	y = 0.66x-11.10	0.27
mono	N	pool	n3 (% lipid)	y = -1.24x+37.20	0.24
18:1	N	pool	18:1 (% DMB)	y = 1.71x+3.80	0.38
18:1	N	pool	18:1 (% lipid)	y = 0.63x-6.81	0.37
18:1	P	low	18:1 (% DMB)	y = 0.56x-0.79	0.58
18:1	P	high	18:1 (% DMB)***	y = 0.23x+0.31	0.06
18:1	N	low	n3 (% DMB)	y = -3.06x+21.51	0.41
18:1	N	high	n3 (% DMB)***	y = -5.85x+36.47	0.23
18:1	N	pool	n3 (% lipid)	y = -1.19x+31.53	0.29
n3	N	pool	n3 (% DMB)	y = 1.29x+0.89	0.44
n3	N	pool	n3 (% lipid)	y = 0.25x+1.16	0.39
n3	P	pool	n3 (% lipid)	y = 0.11x+1.48	0.22
n3 HUFA	N	pool	n3 HUFA (% DMB)	y = 1.25x+0.82	0.46
n3 HUFA	N	pool	n3 HUFA (%lipid)	y = 0.24x+1.00	0.41
n3 HUFA	P	pool	n3 HUFA (%lipid)	y = 0.12x+1.52	0.22
n3 HUFA	N	pool	n3 (% DMB)	y = 1.13x+0.67	0.47
n3 HUFA	N	pool	n3 (% lipid)	y = 0.21x+0.94	0.40
n3 HUFA	P	pool	n3 (% lipid)	y = 0.11x+1.45	0.23
n6	P	low	n6 (% DMB)	y = 3.21x-5.16	0.46
n6	P	high	n6 (% DMB)***	y = 2.27x-4.53	0.40
n6	P	pool	n6 (% lipid)	y = 0.24x-1.52	0.39
n6 HUFA	P	pool	n6 (% lipid)	y = 0.11x-0.93	0.32

*mono = total monounsaturated fatty acids
18:1 = C18 monounsaturated fatty acids
n3 HUFA = n3 highly unsaturated fatty acids (>C18)
n6 HUFA = n6 highly unsaturated fatty acids (>C18)
N = body neutral lipid fraction
P = body polar lipid fraction
**group = treatment group involved in the regression
low = a regression using the low lipid diets (I - V) only
high = a regression using the high lipid diets(VI- X) only
pool = a regression using all diets (I - X)
***This regression did not have slope that was significantly different from zero (P>0.05).

Growth

The weights of the fish at the beginning of the experiment (wt_i), as well as before (wt_g) and after (wt_f) the growth trial are shown in table 18. The data for wt_i corresponds to the initial weight for period 1. The final weight for period 1 is the same as the initial weight for period 2 (wt_g). Wt_f corresponds to the final weight for period 2. The relative growth rate and specific growth rates were calculated for the growth trial (period 2) only. Those values are shown in table 19. Relative growth rate is defined as the final body weight divided by the initial body weight. Similarly, there was no significant difference in the specific growth rate among dietary treatments. Specific growth (SGR) rate has been defined by Yu and Sunnhuber (1979) as follows:

$$SGR = (\log wt_f - \log wt_i)/days$$

where wt_f is final wet body weight and wt_i is initial wet body weight.

Analysis of variance showed that dietary treatments had no significant effect on relative growth rate or specific growth rate. Regression analysis was performed on relative growth rate and specific growth rate as functions of dietary n3 (% DMB and % lipid).

The slopes from the regressions of relative growth rate as functions of dietary n3 (% DMB or % lipid) were not significant.

The high and low lipid groups could not be compared in terms of specific growth rate because the differences in residual variances meant that the high and low lipid groups were heterogeneous.

Simply stated, neither the dietary total lipid content nor the fatty acid composition of the diet significantly affected growth.

Table 18

The mean body weight of fish at the beginning of period 1, between periods 1 and 2, and at the end of period 2.

Diet	Rep	Wt_i* ± SD**	Wt_g ± SD	Wt_f ± SD
I	1	9.49 ± 1.04	7.78 ± 3.02	21.52 ± 14.90
	2	9.61 ± 0.97	7.94 ± 2.87	29.25 ± 19.39
II	1	9.37 ± 1.09	7.01 ± 2.32	24.63 ± 18.78
	2	9.54 ± 0.93	8.26 ± 3.32	29.31 ± 16.03
III	1	9.59 ± 1.14	7.95 ± 2.51	25.29 ± 11.06
	2	9.13 ± 0.95	6.19 ± 1.40	14.63 ± 9.36
IV	1	9.43 ± 0.86	6.77 ± 1.66	15.76 ± 9.82
	2	9.54 ± 1.13	6.85 ± 1.61	18.39 ± 10.67
V	1	9.29 ± 0.96	6.82 ± 1.62	19.45 ± 11.89
	2	9.56 ± 1.05	6.85 ± 2.43	24.20 ± 16.61
VI	1	9.20 ± 0.97	7.90 ± 3.02	25.62 ± 18.97
	2	9.71 ± 1.06	8.20 ± 3.55	24.00 ± 17.92
VII	1	9.73 ± 1.02	6.90 ± 2.25	20.20 ± 11.84
	2	9.27 ± 0.98	6.91 ± 2.53	21.37 ± 15.15
VIII	1	9.70 ± 0.93	8.27 ± 2.93	25.49 ± 14.07
	2	9.56 ± 1.04	7.53 ± 3.05	26.87 ± 15.65
IX	1	9.45 ± 0.91	7.93 ± 3.17	22.26 ± 14.84
	2	9.38 ± 0.92	6.90 ± 2.35	21.46 ± 11.72
X	1	9.41 ± 1.10	6.29 ± 2.31	19.12 ± 12.27
	2	9.47 ± 1.10	7.07 ± 2.91	17.58 ± 14.31

* Wt_i = beginning of period 1 = October 28/88
Wt_g = interface of period 1 and 2 = April 21/89
Wt_f = end of period 2 = July 13/89
** SD = one standard deviation

Table 19.

The number of fish/tank at the beginning of period 1, between periods 1 and 2, and at the end of period 2 as well as the mortality, relative growth rate and specific growth rate observed during period 2.

Diet	Rep	N_i	N_g	N_f	mortality (N_g-N_f)	Relative growth Rate	Specific growth Rate
I	1	60	55	33	22	2.77	0.44
	2	60	55	26	29	3.68	0.57
II	1	60	56	22	34	3.51	0.55
	2	60	55	37	18	3.55	0.55
III	1	60	55	34	21	3.18	0.50
	2	60	55	15	40	2.36	0.37
IV	1	60	56	19	37	2.33	0.38
	2	60	55	26	29	2.68	0.43
V	1	60	56	27	29	2.85	0.45
	2	60	55	12	43	3.53	0.55
VI	1	60	53	29	24	3.24	0.51
	2	60	55	31	24	2.93	0.47
VII	1	60	55	20	35	2.93	0.47
	2	60	54	19	35	3.09	0.49
VIII	1	60	55	31	24	3.08	0.49
	2	60	55	27	28	3.57	0.55
IX	1	60	56	31	25	2.81	0.45
	2	60	54	20	34	3.11	0.49
X	1	60	55	13	42	3.04	0.48
	2	60	55	22	33	2.48	0.40

Mortality

The numbers of fish per tank at different times of the experiment and the respective number of mortalities are listed in table 19. Statistical analysis using a Chi-square test showed that the number of mortality did differ significantly (P>0.05) among treatments. There were significant slopes produced in regressions of recorded mortality functions of dietary n3 (% DMB or % lipid). The regressions are shown in figures 43 and 44, respectively. The r^2 values for these regressions were 0.27 and 0.22 respectively.

It is evident from the regression that increased mortality can be associated with increased dietary n3 fatty acid content. However, it should be noted that the changes in dietary n3 are only responsible for approximately one quarter of the differences in mortality.

The only independent varuable which had a higher r^2 value was the n3 highly unsaturated fatty acids. The r^2 values were 0.28 and 0.24 for n3 HUFA (% DMB) and n3 HUFA (% lipid) respectively. These values might have been expected knowing that the total dietary n3 is comprised of 85 % n3 highly unsaturated fatty acids.

The mortality was due to anorexia or complications of

anorexia. The correlation of dietary fatty acid content to mortality might be more accurately expressed as a correlation of ingestive response to dietary fatty acid content. Unfortunately, the lack of intake data forces speculation on this correlation rather than numerical proof.

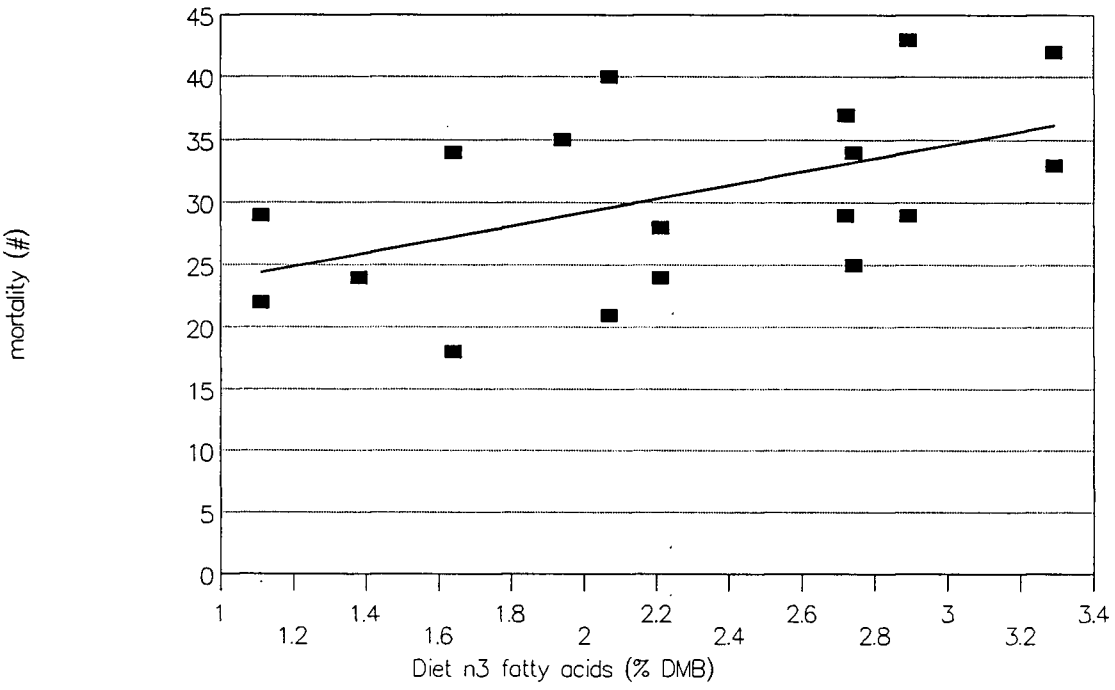

Figure 43. Mortality during period 2 as a function of dietary n3 fatty acids (% DMB).
The regression involves all diets (I-X).
$y = 5.41x + 18.40$ $r^2 = 0.27$

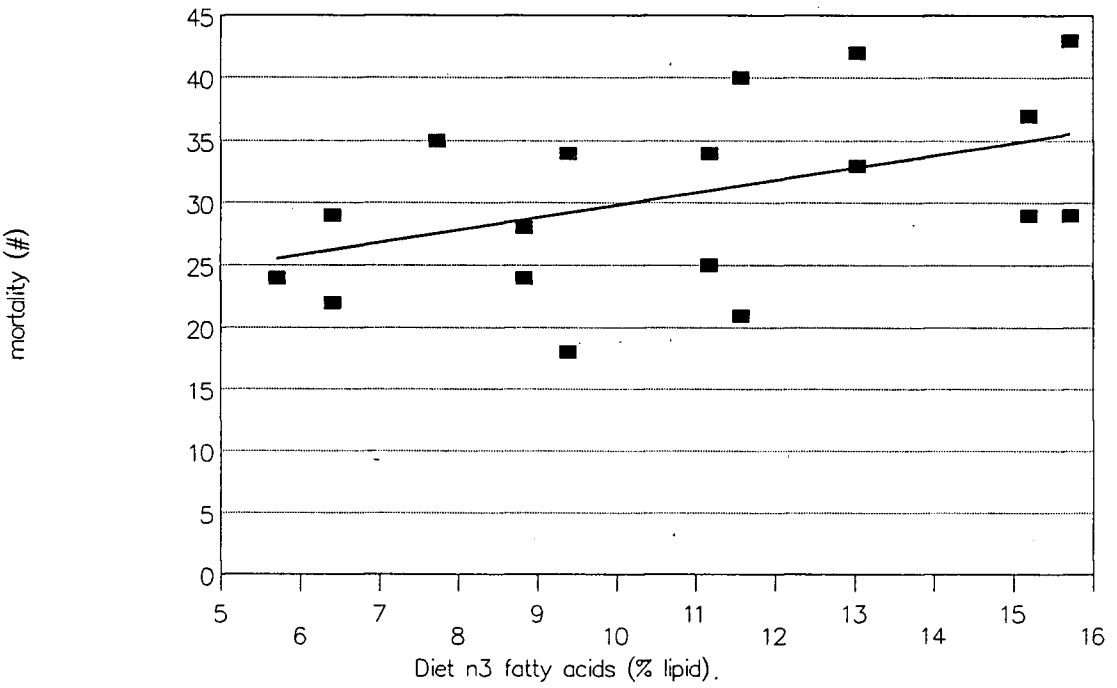

Figure 44. Mortality during period 2 as a function of dietary n3 fatty acids (% lipid).
The regression involves all diets (I-X).
$y = 1.00x + 19.78$ $r^2 = 0.22$

Saltwater tolerance

A. Body composition with respect to saltwater challenge

I. Muscle moisture

There was no significant difference in the dry matter content of muscle among treatments before or after a 24 hour salt-water challenge. It should be noted that the muscle dry matter concentration was affected by the saltwater challenge. The means of the differences (post minus pre-saltwater challenge) did not differ significantly between treatments. The data are shown in table 20.

Table 20.

Percentage dry matter in muscle of coho salmon before and after a 24 hour saltwater challenge.

Diet	Rep	Pre-Challenge muscle dry matter %	Mean	Post-Challenge muscle dry matter %	Mean	Mean Difference
I	a	22.19	22.53	23.09	23.18	0.65
	b	22.87		23.28		
II	a	22.87	22.62	23.54	23.19	0.57
	b	22.38		22.84		
III	a	21.45	21.47	22.86	22.81	1.34
	b	21.49		22.77		
IV	a	21.99	22.03	23.44	23.13	1.10
	b	22.08		22.82		
V	a	22.23	21.46	23.26	22.94	1.48
	b	20.70		22.62		
VI	a	22.42	22.80	24.80	24.64	1.84
	b	23.19		24.48		
VII	a	22.28	21.26	23.44	23.96	2.70
	b	20.25		24.49		
VIII	a	22.76	22.74	23.12	23.46	0.72
	b	22.73		23.80		
IX	a	21.99	22.08	24.71	24.14	2.06
	b	22.18		23.57		
X	a	21.45	22.03	23.03	23.39	1.36
	b	22.61		23.76		

II. Plasma Sodium

The mean plasma Na concentrations for the dietary treatments ranged from 138 - 158 mmol/L. These levels suggest that all dietary treatments produced saltwater-tolerant fish. The data is shown in table 21.

Table 21

Mean Plasma Sodium Concentration (meq/l) following a 24 hour saltwater challenge

Diet	Plasma Na (meq/l) ± SD*
I	157.7 ± 9.2
II	158.8 ± 8.2
III	145.1 ± 6.1
IV	138.3 ± 20.8
V	141.6 ± 22.0
VI	153.0 ± 12.5
VII	152.0 ± 11.1
VIII	157.0 ± 15.7
IX	158.7 ± 12.6
X	149.0 ± 18.6

*SD = standard deviation

B. Mortality

No mortality occurred as a result of the saltwater challenge. All of the fish seemed healthy and active following the 24 hour challenge.

Discussion

Method of quantifying fatty acids in the diet. (% DMB vs % lipid)

The standard method of quantifying dietary fatty acids is as a percent of the diet on a dry matter basis. The essential fatty acids are most often quantified in this manner. Obviously, one fatty acid group, such as n3 fatty acids, only makes up a fraction of the total dietary lipid. This simple statement was the basis for studying whether the response of fish to graded dietary levels of essential fatty acid (n3) is affected by the dietary lipid concentration. The experimental diets were formulated to to duplicate five levels of n3 fatty acid, as a percent of the dry diet, in the low and high-dietary lipid diets. The triolein, however, was an unexpected source of linoleic and linolenic acids. The higher lipid level in diets VI - X was due exclusively to additional triolein. It is for this reason that diets VI - X have higher concentrations of 18:2n6 and 18:3n3 than diets I - V.

The r^2 values for regressions of body fatty acid composition as a function of dietary fatty acids, quantified as percent dry diet (% DMB) or percent dietary lipid (% lipid), did not differ markedly. However, a

comparison of regressions in tables 11 and 17 shows that the r^2 values were consistently higher for the pooled regressions which quantified the independent variable using the percent of dry diet (% DMB) rather than using the percent of the dietary lipid (% lipid). This suggests that relating body fatty acid composition to dry diet fatty acid concentration (% DMB) is only slightly more accurate than is relating it to dietary lipid fatty acid concentration (% lipid). The magnitude of the differences does not justify emphasizing one method of quantifying dietary fatty acids over the other.

The effect of dietary lipid concentration on the relationship between dietary fatty acids and body fatty acid composition.

Two total lipid concentrations were used in this experiment to create the different ratios of fatty acid group to diet (% DMB or % lipid). Altering dietary lipid levels leads to complications in understanding the metabolism and deposition of dietary fatty acids. Dietary intake and its association to energy in the diet affect the experiment.

Altering the total dietary lipid concentration confounds a study profoundly. One problem lies in the effect of increased lipid on digestible energy levels in

the diet. The low (diets I-V) and high (diets VI-X) lipid diets in this study were not isocaloric. This dietary difference dictates that the principle of dietary intake as a function of dietary energy level be considered.

It is believed that fish base their food intake on energy intake. Fish fed a low total lipid diet consume more, on a dry matter basis, than they would on a high lipid diet (Lee and Putnam, 1973) and (Page and Andrews, 1973). If a low lipid diet and high lipid diet had the same amount of a certain fatty acid group (% DMB), for example n3, then the fish consuming the lower lipid diet should be ingesting more grams of n3 fatty acids for a given body weight.

However, if intake does not differ with changes in the total dietary lipid concentration then fish consuming the high lipid diet would consume more dietary lipid. Observations relating dietary fatty acid to body fatty acid content at different dietary lipid levels rely on an understanding of what effect the dietary lipid has on growth and total lipid composition of the body. Is the dietary lipid being catabolized for energy thereby resulting in growth or is it being stored in the body?

The fish which were fed the high lipid diets in this experiment showed neither a significant increase in body

lipid nor a significant increase in growth when compared to those fed the low lipid diet. These contradictory results necessitate evaluation of the actual ingestive response of the fish to determine what is occurring within the fish. Unfortunately, the poor response to the diet and the environment by the experimental animals made such data unobtainable.

Fortunately, the fact that there was statistical justification for pooling high and low lipid dietary groups into one regression during the fatty acid analysis makes the statement that body fatty acid composition is affected by dietary fatty acid composition in a consistent manner regardless of the total lipid content of the diet. This statement holds true for both the polar and neutral body lipid pools and was consistent in the period 1 and period 2 samples.

The effect of dietary fatty acids on body fatty acid composition

The interactions between dietary fatty acid content and body fatty acid composition were not consistent in the two samples of fish (periods 1 and 2). Differences in body fatty acid composition between the period 1 samples and the period 2 samples are difficult to interpret.

These samples of fish, although from the same populations, were of different body weights, body lipid content, and neutral lipid content. The difference in age of these two groups was 98 days. It must be noted that the period 1 sample were taken from 5° C. water. They had been raised at between 3 - 5 ° C. for 61 days. The period 2 sample was taken from a 15° C. environment. The growth trial (period 2) began at 7.5 ° C. It took 33 days for the ambient temperature to rise to 10° C. Following this the temperature was heated one degree per day for two days and then remained consistently at 12° C. for 7 days. The temperature was again elevated by one degree per day to 14.5° C. where it remained for the remaining 39 days of the growth trial.

Although there was an obvious difference in mean environmental (temperature) between periods 1 and 2 associating the differences in fatty acid composition exclusively with water temperature may lead to erroneous conclusions. The differences in age, size, physical state and body composition were substantial. The data in tables 6 and 12 show considerable differences in total, polar and neutral lipid concentrations in the body tissues. The age difference may influence hormonal activity while the body size and physical state may relate to the activity of the

fish. For the relationship of water temperature and dietary fatty acid effect to be properly addressed the dietary treatments should be administered simultaneously to a homogeneous population of fish at the different water temperatures.

It was the period 1 fish which displayed an effect of diet on body saturated fatty acid content. The increases in saturate content in the diet seemed to be slightly more responsible for the decreases in neutral saturates in the body than were the increases in dietary n3 as is seen in table 11. This trend was also noted in a report by Castledine and Buckley (1980). However, the dietary effect was not repeated in the period 2 sample in this experiment. Table 4 shows that the dietary saturate concentration increased when dietary n3 fatty acids increased. The n3 fatty acids in the dietary treatments increased at nearly twice the rate in which saturates increased. This relationship of saturated and n3 fatty acids might have been expected to produce a positive effect on neutral body saturated fatty acids rather than the negative relationship which occurred. Dietary monounsaturated fatty acids, including 18:1, also affected the neutral saturate content in the body. Increased dietary monounsaturated fatty acids caused saturated fatty

acids in the body neutral lipid to increase. Table 4 shows that the monounsaturated fatty acids in the diet increased as dietary n3 and saturated fatty acids decreased. Reviewing the regressions and r^2 values for these relationships (table 11) shows that the monounsaturated fatty acids are only slightly more responsible for the changes in body neutral saturated fatty acid concentrations than are the dietary n3 fatty acids. It is uncertain what caused the unexpected relationship between dietary and body fatty acids.

The essential fatty acid requirement in the fish remains regardless of low growth rate or metabolic activity. The maintenance of biological membranes within the body is the most probable requirement for these fatty acids. Perhaps the low water temperature caused a decrease in ingestive activity in comparison to period 2 fish. At this low intake level the diet with the lowest n3 fatty acid concentration may not have been sufficient for providing the amount of n3 required for maintenance of the cell membranes and other essential functions. If this was the case, the neutral lipid pool would be serving as a source of n3 fatty acids. As the neutral lipid was depleted of this class of fatty acids the concentrations of other classes of fatty acids in the neutral fraction

would increase. It seems as though this is what has happened in the body with the neutral saturated fatty acid fraction.

This may have been what Castledine and Buckley (1980) observed when they hypothesized that fatty acids are catabolized on a molar basis in the neutral lipid during starvation. It is possible that long-chained highly unsaturated fatty acids in the neutral lipid were being removed and incorporated into the polar lipid as maintenance of the cell membranes occurred.

The lack of a significant relationship between dietary n3 fatty acids and body n3 fatty acids also relates to the above scenario. It seems that in period 1 the body neutral lipid pool was a more important source of n3 fatty acids for the polar lipid than was the dietary lipid.

Perhaps the essential fatty acid requirement of fish should be stated as a function of body weight directly rather than indirectly. By stating the requirement as a function of the diet nutritionists are erroneously implying that dietary intake remains as a constant function of the body weight, as is the case with homeotherms. The metabolic rate and ingestive rate in poikilotherms do change when ambient temperature changes.

However, it is uncertain whether these two rates are innately synchronized to ensure adequate nutrition when the diet does not change. If the lower metabolic rate does not compensate for the lower ingestive rate then the n3 fatty acid requirement, as a function of the diet, may change by a different increment than would the n3 fatty acid requirement as a function of body weight.

Increased dietary monounsaturate concentration consistently resulted in increased monounsaturate content in the body neutral lipid fraction. Similarly, an increase in the dietary n3 content, which corresponded to a decreased dietary monounsaturate content, decreased the monounsaturate fatty acid concentration in the body neutral lipid. This was also observed by Castledine and Buckley (1980). A similar trend occurred in the polar lipid of the period 1 fish. Polar monounsaturate increased with increased dietary monounsaturate and decreased with increased dietary n3 concentrations.

C18 monounsaturated fatty acid concentration in the body neutral lipid fraction was affected positively by dietary C18 concentration and inversely by changes in dietary n3 in both periods. This trend was also observed by Takeuchi and Watanabe (1977a). The monounsaturated fatty acid content in the body polar lipid was affected by

diet only in the period 1 sample. Increased dietary n3 resulted in decreased 18:1 fatty acid content in the body polar lipid.

The effect of n3 fatty acids in the diet on body n3 fatty acids was different for the two sample groups. The period 2 sample showed an increase in n3 fatty acids in the body neutral lipid when dietary n3 was increased. It has already been mentioned that the period 1 body n3 content was not affected by changes in dietary n3 concentration.

n3 highly unsaturated fatty acids (HUFA's) in the body neutral lipid were affected in a similar manner to the total n3 in that lipid fraction. During period 2, fish stored levels of n3 HUFA's in the neutral lipid which corresponded to dietary n3 HUFA and total n3 increases. The neutral lipid in the period 1 fish was not affected by increased dietary n3 HUFA or total n3 levels. n3 HUFA content in the body polar lipid also increases with increases in dietary n3 HUFA or total n3 in the period 2 fish. This relationship was reported by Castledine and Buckley (1980). The polar n3 HUFA concentration in fish during period 2 was again not affected by changes in dietary n3 HUFA or total n3 content.

Increased dietary n6 content consistently increased

n6 content in the body polar lipid in both the period 1 and period 2 fish. In the period 2 fish, n6 HUFA concentration in the body polar lipid increased as dietary n6 levels rose. The increase in n6 polyunsaturated fatty acids in the polar fraction provides further evidence towards the competition of C18 polyunsaturated fatty acids for elongation, desaturation and incorporation into phospholipids. Similar findings were reported by Lee et al. (1967) and Castledine and Buckley (1980).

The issue of competition of 18:1n9, 18:2n6 and 18:3n3 for the elongation and desaturation mechanism is not easily addressed by this study. It is difficult to examine the elongation and desaturation of 18:1 because 16:1n9 and 20:1n9 were also found in the diets in substantial amounts. All of these fatty acids were observed in the body lipids. However, the lack of 20:3n9 suggests that 18:2n6 and 18:3n3 were effective in inhibiting the elongation and desaturation of 18:1n9.

At first glance the 18:3n3 seems to be more effective than 18:2n6 in creating long-chained highly unsaturated fatty acids. Table 4 shows diets III and VIII as having equal concentrations of n3 and n6 fatty acids. The concentrations of highly unsaturated fatty acids in tables 12 - 15 consistently shows n3 to exceed n6 fatty acids in

the body. However, 85% of the total n3 fatty acids in the diet are highly unsaturated. Knowing this, the ratio of n3 HUFA to n6 HUFA in the body lipid is less impressive since 85% of the body n3 HUFA's could have been obtained directly from the diet without elongation or desaturation.

Unfortunately, not much can be said about this issue because of the variety of dietary constituents in this experiment. The issue of elongation and desaturation should be addressed using radioactively labelled 18:1n9, 18:2n6 and 18:3n3 in the diet or by feeding purified diets which only have short-chained fatty acids.

The essential fatty acid index, 20:3n9/22:6n3, reported by Owen et al. (1975) and Takeuchi and Watanabe (1979) could not be addressed in this study because 20:3n9 was not detected in the body lipid. The dietary concentration of n3 and n6 fatty acids probably inhibited the elongation and desaturation of n9 monounsaturated fatty acids in agreement with the findings of Brockerhoff et al. (1966). This relationship was also reported by Yu and Sinnhuber (1972), Takeuchi et al. (1979) and Takeuchi and Watanabe (1977a).

Body moisture content did not seem to be affected by dietary n3 fatty acid concentration. This observation is contrary to one report of a evidence of a negative

relationship (Takeuchi and Watanabe, 1979) and one of a positive relationship (Castell et al., 1972b.).

The effect of dietary n3 fatty acid concentration on growth

The initial poor ingestive response to the diets coupled with the disadvantageous environmental conditions during period 1 resulted in high mortality and much variation in the size of the fish in period 2. The high and variable rates of mortality make the growth rates difficult to evaluate.

The lack of a significant difference in growth agrees with the results of Yu and Sinnhuber (1979) for the dietary n3 fatty acid concentrations between 1.0 and 2.5% (% DMB). Growth in coho salmon is not improved by increased amounts of dietary n3 fatty acids as was observed in rainbow trout (Takeuchi and Watanabe, 1977a). This finding may prove to be important to the aquaculture industry if either the price or the availability of marine fish oils makes the use of this ingredient impractical. However, further research is required to better determine the optimum dietary concentration of n3 fatty acids. A long-term study of the effect of dietary fatty acids on growth and body composition would be very useful in

mapping out the dietary effect over different environmental and physiological conditions.

The effect of dietary n3 fatty acids concentration on mortality

There were little mortality during period 1 of this experiment. During period 2, on the other hand, mortality ranged from 33 - 78 % among tanks. Mortality was associated with anorexia and lethargy. Failure to feed during period 1 meant that the fish had to catabolize body stores for nutrients. The drop in water temperature during period 1 would have reduced metabolic rate in the fish. The low metabolic rate allowed the fish to survive during period 1. However, the metabolic rate would have increased during period 2 when the water temperature increased. This elevation in metabolic rate increased the energy requirement of the fish. At this point in the experiment some of the fish were so debilitated that they did not commence eating and subsequently died when they had depleted their body stores.

It is very likely that the anorexic fish never ate during the whole experiment. It is for this reason that associating the mortality to the ingestive response is more logical than associating it to an effect of dietary fatty acids on body fatty acid composition.

Chi-square analysis showed a significant difference ($P>0.05$) in the mortality among treatments. This effect of dietary fatty acid concentration on mortality was examined by regressions. Increases in dietary n3 and n3 highly unsaturated fatty acids were partially responsible for increases in mortality. The trend of increasing mortality with increasing n3 or n3 HUFA concentration in the diet has not been previously reported in the literature. It is unclear why such a relationship would occur. There have been reports of a negative effect of high dietary n3 concentrations in growth in coho salmon (Yu and Sinnhuber, 1979) and rainbow trout (Takeuchi and Watanabe, 1979).

The small r^2 values for the regressions of mortality as a function of these dietary fatty acid classes suggests that some unknown factor is involved in the abnormally high mortality. Perhaps there was a constituent in the herring oil which was disliked by the fish in this experiment. The lack of accurate intake data limits assessment of the cause of the high mortality.

The effect of dietary n3 fatty acid concentration on saltwater tolerance

There was no mortality as a result of a 24 hour

challenge in full strength artificial sea water. The fish were equally able to withstand the direct immersion into saltwater regardless of dietary treatment.

There was also no significant difference in the dry moisture content of muscle prior to or following the 24 hour challenge as was the case prior to the challenge. The analysis of variance of the mean dry matter content (post saltwater challenge minus freshwater) showed no significant difference between dietary treatments. This observation suggests that the differences in polar fatty acid concentration, due to the diet, do not impair the effectiveness of the biological membranes in maintaining osmosis.

The mean treatment plasma sodium concentrations (post-saltwater challenge) did not exceed the 170 meq/l. This concentration has been stated by Clarke and Blackburn (1977) as evidence of saltwater tolerance.

It appears that dietary n3 fatty acid concentration does not affect saltwater tolerance in juvenile coho salmon. This finding is in agreement with Markert et al. (1984) and Plotnikoff et al. (1983). The coho in this experiment were 1+ years old. Under most conditions these fish would undergo transfer to saltwater at this age. It is important that the dietary treatment did not impede the

ability of the fish to undergo this critical physiological change in their life.

Conclusion

Body concentrations of total lipid, polar lipid and neutral lipid were not affected by dietary lipid content or dietary fatty acid composition. The composition of polar and neutral lipids was significantly altered by dietary fatty acid concentration. Polar lipid composition was, however, comparatively stable relative to changes in dietary fatty acids. The response of body lipids to dietary fatty acids appeared to be modified by water temperature. However, cognizance must be taken of the difference between the samples of fish. As a result of feed consumption in the fish during period 2, the fish had gained weight and accumulated body lipid.

The n6 fatty acid concentration in the body polar lipid consistently increased with increases in dietary n6 content. The period 1 fish showed that polar monounsaturate concentration corresponded directly with dietary monounsaturate content and inversely with dietary n3 content. During period 2, n3 levels in the polar lipid of the fish increased with higher dietary concentrations of n3 fatty acids. Higher n3 HUFA concentrations in the polar lipid were likewise increased when dietary n3 or n3 HUFA content increased. During period 2, increases in

dietary n6 fatty acids also increased n6 HUFA levels in the body polar lipid when dietary n6 levels increased. The changes in the polar lipid fatty acid profile may be attributed to the competitive elongation and desaturation between 18:1n9, 18:2n6 and 18:3n3 to their respective long-chained highly unsaturated fatty acids. These three families of fatty acids compete for the same enzymatic mechanism for elongation and desaturation. This mechanism is substrate concentration dependent. The fact that 20:3n9 was not detected in body lipid strongly suggests that dietary 18:3n3 and 18:2n6 inhibit elongation and desaturation of 18:1n9. The differences in dietary concentrations of 18:2n6 and 18:3n3 within the experimental diets make it difficult to evaluate the inhibitive effect of these fatty acids upon each other.

Total monounsaturated and 18:1 monounsaturated fatty acid concentration in the body corresponded directly with the respective concentration of dietary total monounsaturated or 18:1 fatty acids. Each of these fatty acid groups were decreased in the neutral lipid by increases in dietary n3. Saturated fatty acid content in the body neutral lipid decreases with increases in dietary n3 or saturated fatty acids in the period 1 fish. However, this relationship was probably the result of

inadequate intake of essential fatty acids and the subsequent transfer of n3 fatty acids from the body neutral lipid pool to the polar. n3 HUFA concentrations in the body neutral lipid increased during period 2 when feeding occurred and diets containing the higher concentrations of total n3 or n3 HUFA were fed.

The fatty acid composition of body lipids reacted similarly to changes in fatty acid content regardless of the manner in which the diet content was quantified, percent of the dry diet or percent of the dietary lipid. It seems that dietary nutrient concentrations in poikilotherms should be stated as absolute amounts ingested rather than amounts in the diet or in a dietary component such as lipid. This is because nutrient requirements may be maintained regardless of metabolic rate resulting in inadequate nutrition when activity and food ingestion decreases at low temperatures.

The growth response was difficult to interpret because of the high mortality. Mortality during period 2 increased as total n3 or n3 HUFA content in the diet increased. The poor appetitive response is probably more responsible for the mortality than is dietary fatty acid content. Saltwater tolerance was not impeded by changes in the dietary treatment. All dietary treatments showed

evidence of the ability to effectively undergo transfer to full strength sea water.

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